

PCT

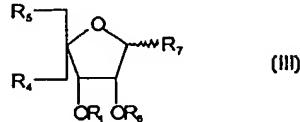
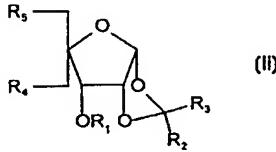
WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ :	A2	(11) International Publication Number: WO 00/56746						
C07H 19/00		(43) International Publication Date: 28 September 2000 (28.09.00)						
<p>(21) International Application Number: PCT/DK00/00141</p> <p>(22) International Filing Date: 24 March 2000 (24.03.00)</p> <p>(30) Priority Data:</p> <table> <tr> <td>PA 1999 00407</td> <td>24 March 1999 (24.03.99)</td> <td>DK</td> </tr> <tr> <td>PA 2000 00099</td> <td>21 January 2000 (21.01.00)</td> <td>DK</td> </tr> </table> <p>(71) Applicant (for all designated States except US): EXIQON A/S [DK/DK]; Bygstubben 9, DK-2950 Vedbæk (DK).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): KOCHKINE, Alexei [DK/DK]; c/o Jørgensen, Borgergade 26, apartment 36, DK-1300 Copenhagen K (DK). FENSHOLDT, Jef [DK/DK]; Årtemarken 79, DK-2860 Søborg (DK). PFUNDHELLER, Henrik, M. [DK/DK]; Dalgas Have 2, 4. tv., DK-2000 Frederiksberg (DK).</p> <p>(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Anna Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).</p>		PA 1999 00407	24 March 1999 (24.03.99)	DK	PA 2000 00099	21 January 2000 (21.01.00)	DK	<p>(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, DZ, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (Utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p>
PA 1999 00407	24 March 1999 (24.03.99)	DK						
PA 2000 00099	21 January 2000 (21.01.00)	DK						

(54) Title: IMPROVED SYNTHESIS OF [2.2.1]BICYCLO NUCLEOSIDES



(57) Abstract

A synthesis of [2.2.1]bicyclo nucleosides which is shorter and provides higher overall yields proceeds via the key intermediate of general formula (III), wherein R₄ and R₅ are, for instance, sulfonates and R₇ is, for instance, a halogen or an acetate. From compounds in general formula (II), such as 3-O-aryl-4-C-hydroxymethyl-1,2-O-isopropylidene- α -D-ribofuranose, intermediates of general formula (III) are suitable for coupling with silylated nucleobases. Upon one-pot base-induced ring-closure and desulfonation of the formed [2.2.1]bicyclo nucleoside, a short route to each the LNA (Locked Nucleic Acid) derivatives of adenosine, cytosine, uridine, thymidine and guanine is demonstrated. The use of the 5'-sulfonated ring-closed intermediate also allows for synthesis of 5'-amino- and thio-LNAs.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

IMPROVED SYNTHESIS OF [2.2.1]BICYCLO NUCLEOSIDES

FIELD OF THE INVENTION

- 5 The present invention relates to a new strategy for the synthesis of [2.2.1]bicyclo nucleosides which is shorter, provides higher overall yields, and thus more cost efficient than previously known methods for synthesis of [2.2.1]bicyclo nucleosides.

BACKGROUND OF THE INVENTION

10

Synthesis of the LNA (Locked Nucleic Acid) monomer (1S, 3R, 4R, 7S)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane uracil was first reported by Obika. (Satashi Obika et al., *Tetrahedron Lett.*; 1997; 8735-8738) who used a linear strategy based on uridine as starting material for the synthesis of the intermediate 1-(3-O-benzyl-4-C-

15 tosyloxymethyl- β -D-ribofuranosyl)uridine. Treatment of the tosylated nucleoside intermediate with sodium hexamethyldisilazide in THF afforded the 2'-O,4'-C-methylene bicyclonucleoside which upon final debenzylation afforded (1S, 3R, 4R, 7S)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane uracil in 36% yield from the tosylated nucleoside intermediate.

20

Wengel et al. (Singh, S. K.; Nielsen, P., Koshkin, A. A. and Wengel, J., *Chem. Commun.*, 1998, 455; Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Melgaard, M.; Olsen, C. E. and Wengel, J., *Tetrahedron*, 1998, 54, 3607) subsequently reported on a convergent strategy for the synthesis of the thymine analogue (1S, 3R, 4R,

25 7S)-7-hydroxy-1-hydroxymethyl-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane. Starting from 3-O-benzyl-4-C-hydroxymethyl-1,2-O-isopropylidene- α -D-ribofuranose, the key intermediate for coupling with silylated thymine (or other silylated nucleobases), 4-C-acetoxyethyl-1,2-di-O-acetyl-3,5-di-O-benzyl-D-ribofuranose, was obtained by successive regioselective 5-O-benzylation, acetylation, acetolysis, and another

30 acetylation. Coupling of the key intermediate with silylated thymine afforded the 4'-C-acetoxyethyl nucleoside which upon deacetylation and monotosylation followed by base-induced ring closure, afforded the 2'-O,4'-C-methylene bicyclonucleoside. Final debenzylation gives (1S, 3R, 4R, 7S)-7-hydroxy-1-hydroxymethyl-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane in 40% yield (calculated from the key intermediate). Analogous 35 synthetic procedure were applied for the synthesis of the uracil, 2-N-isobutyrylguanine, 4-

- N*-benzoylcytosine and 6-*N*-benzoylcytosine LNA nucleoside analogues. The corresponding 2'-amino-LNA pyrimidine nucleosides were obtained by performing the ring closure in benzylamine. Debenylation and subsequently silylation using 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane afforded a bicyclic intermediate which was easily 5 converted into the 2'-thio-LNA analogue upon reaction with potassium thioacetate in DMF and final desilylation (Singh, S. K.; Kumar, R. and Wengel, J., *J. Org. Chem.*, 1998 63, 6078).

An analogous convergent synthesis of the (1*S*, 3*R*, 4*R*, 7*S*) -7-hydroxy-1-hydroxymethyl-10 2,5-dioxabicyclo[2.2.1]heptane thymine using 4-C-tosyloxymethyl-1,2-di-O-acetyl-3,5-di-O-benzyl-D-ribofuranose as the key intermediate for coupling with silylated nucleobases has been reported by the same group (Koshkin, A. A., Rajwanshi, V. K., and Wengel J., *Tetrahedron Lett.*, 1998, 39, 4381).

15 The use of a 4-C-tosyloxymethyl ribofuranose intermediate has also been suggested by Obika, S. et al (WO 98/39352). In this strategy the 5-O-benzyl protecting group is exchanged for a *tert*-butyldimethylsilyl protecting group thereby extending the total synthesis of (1*S*, 3*R*, 4*R*, 7*S*)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane nucleosides with one step.

20

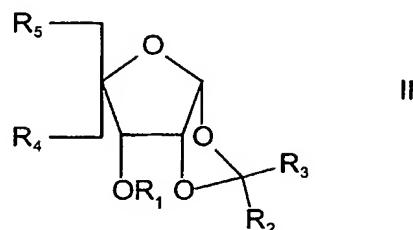
Characteristic properties of the previously known strategies discussed above are relatively low overall yields and many synthetic steps. Thus, there is a great need for development of a more efficient synthesis strategy which will result in an improvement of the overall yield and a reduction in the production costs of [2.2.1]bicyclo nucleosides.

25

SUMMARY OF THE INVENTION

The present invention provides a novel strategy for the synthesis of [2.2.1]bicyclic nucleosides comprising the synthesis of a novel key intermediate. The novel strategy is 30 demonstrated by the synthesis of (1*S*, 3*R*, 4*R*, 7*S*)-7-hydroxy-1-hydroxymethyl-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane and has easily been extended to the synthesis of [2.2.1]bicyclo nucleosides containing other nucleobases and can be further extended to other heteroatoms than oxygen in the bicycle, such as amino and thio.

The present invention relates to a method for the synthesis of a novel intermediate of the general formula II:



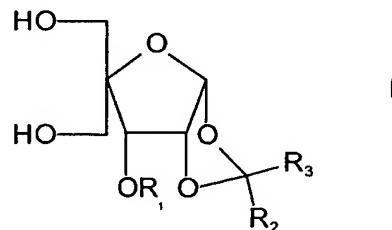
wherein R₁ is selected from optionally substituted aryl(C₁₋₆-alkyl), optionally substituted tetrahydropyran-2-yl, optionally substituted arylcarbonyl and optionally substituted aryl;

each of the substituents R₂ and R₃ is independently selected from hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted aryl, and optionally substituted aryl(C₁₋₆-alkyl), with the proviso that R₂ and R₃ are not both hydrogen, or R₂ and R₃ together designate C₃₋₇-alkylene; and

each of the substituents R₄ and R₅ independently is R'SO₂O- wherein R' is selected from optionally substituted alkyl and optionally substituted aryl;

said method comprising the following step:

treatment of a compound (hereinafter termed "starting material") of the general formula I:



wherein R₁ is selected from optionally substituted aryl(C₁₋₆-alkyl), optionally substituted tetrahydropyran-2-yl, optionally substituted arylcarbonyl and optionally substituted aryl;

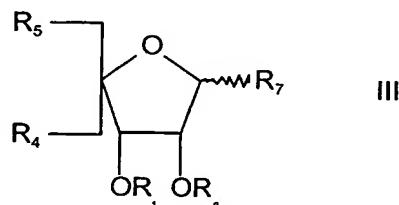
each of the substituents R₂ and R₃ is independently selected from hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted aryl, and optionally substituted aryl(C₁₋₆-alkyl),

with the proviso that R₂ and R₃ are not both hydrogen, or R₂ and R₃ together designate C₃₋₇-alkylene; and

with R'SO₂X wherein R' is selected from optionally substituted C₁₋₆-alkyl and optionally substituted aryl, and X designates halogen.

The present invention also relates to the compound of the general formula II as defined above.

- 10 The present invention furthermore relates to the compound (hereinafter termed "key intermediate") of the general formula III:



wherein R₁ is selected from optionally substituted aryl(C₁₋₆-alkyl), optionally substituted tetrahydropyran-2-yl, optionally substituted arylcarbonyl and optionally substituted aryl;

each of the substituents R₄ and R₅ independently is R'SO₂O- wherein R' is selected from optionally substituted alkyl and optionally substituted aryl;

20 R₆ is selected from hydrogen, optionally substituted (C₁₋₆-alkyl)carbonyl, optionally substituted arylcarbonyl, optionally substituted aryl(C₁₋₆-alkyl), optionally substituted C₁₋₆-alkyl, and tri(alkyl/aryl)silyl; and

R₇ is selected from optionally substituted (C₁₋₆-alkyl)carbonyloxy, optionally substituted C₁₋₆-alkoxy, halogen, optionally substituted arylthio, optionally substituted C₁₋₆-alkylthio, and optionally substituted aryloxy.

The main advantages of the present invention comprise the following:

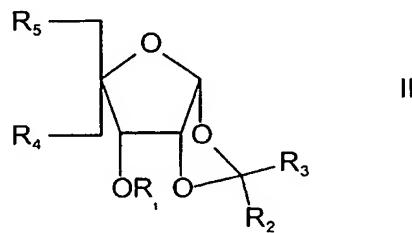
- Obtaining the key intermediate of the general formula III ready for coupling with silylated nucleobases in very few steps from 3-O-benzyl-4-C-hydroxymethyl-1,2-O-isopropylidene- α -D-ribofuranose.
- 5 ▪ One-pot base-induced ring-closure and desulfonation of the formed [2.2.1]bicyclo nucleoside.
- The possibility of using the 5'-sulfonated ring-closed intermediate (compound 5a in example 4) for synthesis of 5'-amino- and thio-LNA.

10

DETAILED DESCRIPTION OF THE INVENTION

In an attempt to improve the synthesis of [2.2.1]bicyclo nucleosides, a novel key intermediate for coupling with different nucleobases was synthesised. Using this novel synthesis strategy comprising the novel key intermediate of the general formula III, (1S, 3R, 4R, 7S)-7-hydroxy-1-hydroxymethyl-(thymin-1-yl)-2,5-dioxabicyclo [2.2.1]heptane was synthesised in only five steps from 3-O-benzyl-4-C-hydroxymethyl-1,2-O-isopropylidene- α -D-ribofuranose, which makes the novel strategy at least two synthetic step shorter than any previously known strategy. The reduction in numbers of synthetic steps as well as the fact that no chromatographic separation of isomers and fewer deprotection steps are required makes the novel synthesis more convenient and much more cost efficient than previously known strategies. This novel synthesis strategy comprising the novel key intermediate of the general formula III also provided surprisingly facile access to [2.2.1]bicyclo nucleosides comprising other nucleobases and to intermediates which are amenable to oligomerization.

The present invention relates to a method for the synthesis of a novel intermediate with the general formula II:



wherein R₁ is selected from optionally substituted aryl(C₁₋₆-alkyl), optionally substituted tetrahydropyran-2-yl, optionally substituted arylcarbonyl and optionally substituted aryl.

Some preferred embodiments comprise benzyl, *o*-, *m*-, and *p*-methylbenzyl, 2-

- 5 chlorobenzyl, 4-phenylbenzyl, tetrahydropyran-2-yl, benzoyl, phenyl, among which benzyl and 4-phenylbenzyl are preferred; and

each of the substituents R₂ and R₃ independently is selected from hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted aryl, and optionally substituted aryl(C₁₋₆-alkyl).

- 10 with the proviso that R₂ and R₃ are not both hydrogen, such as methyl, trifluoromethyl, ethyl, propyl, *iso*-propyl, butyl, *t*-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, phenyl, benzyl, phenylethyl, *o*-, *m*-, and *p*-methylbenzyl, 2-chlorobenzyl, or R₂ and R₃ together designate C₃₋₇-alkylene, such as 1,3-propylene, 1,4-butylene, 1,5-pentylene; and

- 15 each of the substituents R₄ and R₅ independently is R'SO₂O-, wherein R' is selected from optionally substituted C₁₋₆-alkyl, optionally substituted aryl, and optionally substituted aryl(C₁₋₆-alkyl), such as methyl, trifluoromethyl, ethyl, 2,2,2-trifluoroethyl, propyl, *iso*-propyl, butyl, nonafluorobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, benzyl, *o*-, *m*- or *p*-methylbenzyl, 2-chlorobenzyl, phenyl, *o*-, *m*- or *p*-bromophenyl, and *p*-nitrophenyl.

20

In a preferred embodiment of the invention, the substituents R₂ and R₃ independently represent hydrogen, methyl, phenyl, benzyl, phenylethyl, preferably methyl.

- In an even more preferred embodiment of the invention, the substituents R₂ and R₃ both
25 represent methyl.

In another embodiment of the invention, each of the substituents R₄ and R₅ represent methanesulfonyl, trifluoromethanesulfonyl, ethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, propanesulfonyl, *iso*-propanesulfonyl, butanesulfonyl, nonafluorobutanesulfonyl,

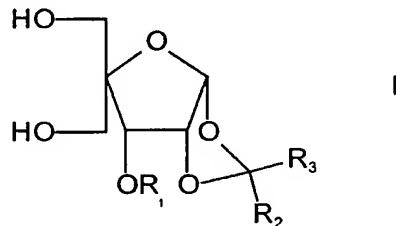
- 30 pentanesulfonyl, cyclopentanesulfonyl, hexanesulfonyl, cyclohexanesulfonyl, *o*-toluenesulfonyl, 2-chloro-*o*-toluenesulfonyl, *o*-, *m*-, *p*-toluenesulfonyl, benzenesulfonyl, *o*-, *m*-, *p*-bromobenzenesulfonyl, and *o*-, *m*-, *p*-nitrobenzenesulfonyl, preferably methanesulfonyl, trifluoromethanesulfonyl, *p*-toluenesulfonyl and *p*-bromobenzenesulfonyl, more preferably methanesulfonyl, and *p*-toluenesulfonyl, even
35 more preferably methanesulfonyl.

In a preferred embodiment of the invention, R₄ and R₅ represent methanesulfonyl, trifluoromethanesulfonyl, ethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, butanesulfonyl, nonafluorobutanesulfonyl, α -toluenesulfonyl, *p*-toluenesulfonyl, benzenesulfonyl, *p*-bromobenzenesulfonyl, and *p*-nitrobenzenesulfonyl, preferably methanesulfonyl, trifluoromethanesulfonyl, *p*-toluenesulfonyl and *p*-bromobenzenesulfonyl, more preferably methanesulfonyl, and *p*-toluenesulfonyl, even more preferably methanesulfonyl.

In an especially preferred embodiment of the invention, R₄ and R₅ are identical and are selected from methanesulfonyl, trifluoromethanesulfonyl, ethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, butanesulfonyl, nonafluorobutanesulfonyl, α -toluenesulfonyl, *p*-toluenesulfonyl, benzenesulfonyl, *p*-bromobenzenesulfonyl, and *p*-nitrobenzenesulfonyl, preferably methanesulfonyl, trifluoromethanesulfonyl, *p*-toluenesulfonyl and *p*-bromobenzenesulfonyl, more preferably methanesulfonyl, and *p*-toluenesulfonyl, even more preferably methanesulfonyl.

Said method comprising the following step:

treatment of a compound with the general formula I:



20

wherein R₁, R₂ and R₃ are as defined above;

with R'SO₂X (hereinafter "sulfonyl halide(s)") wherein R' is selected from optionally substituted C₁₋₆-alkyl, optionally substituted aryl, and optionally substituted aryl(C₁₋₆-alkyl), such as methyl, trifluoromethyl, ethyl, 2,2,2-trifluoroethyl, propyl, *iso*-propyl, butyl, nonafluorobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, benzyl, *o*-, *m*- or *p*-methylbenzyl, 2-chlorobenzyl, phenyl, *o*-, *m*- or *p*-bromophenyl, *p*-nitrophenyl, and X designates halogen, such as fluoro, chloro, bromo, and iodo.

30 In a preferred embodiment of the invention, R₁ represent benzyl.

In another preferred embodiment of the invention, R₂ and R₃ is selected from methyl, ethyl, propyl, *iso*-propyl, benzyl, phenylethyl, phenyl, or R₂ and R₃ together designate 1,3-propylene, 1,4-butylene, and 1,5-pentylene.

5

In a more preferred embodiment of the invention, R₂ and R₃ both represent methyl.

In an especially preferred embodiment of the invention, R₁ represent benzyl and R₂ and R₃ both represent methyl.

10

In a preferred embodiment of the invention, R'SO₂X represents sulfonyl halides, such as methanesulfonyl chloride, trifluoromethanesulfonyl chloride, ethanesulfonyl chloride, 2,2,2-trifluoroethanesulfonyl chloride, propanesulfonyl chloride, *iso*-propanesulfonyl chloride, butanesulfonyl chloride, nonafluorobutanesulfonyl chloride, cyclopentanesulfonyl

- 15 chloride, hexanesulfonyl chloride, cyclohexanesulfonyl chloride, α -toluenesulfonyl chloride, *p*-toluenesulfonyl chloride, *p*-bromobenzenesulfonyl chloride, *p*-nitrobenzenesulfonyl chloride, preferably methanesulfonyl chloride, trifluoromethanesulfonyl chloride, ethanesulfonyl chloride, 2,2,2-trifluoroethanesulfonyl chloride, nonafluorobutanesulfonyl chloride, α -toluenesulfonyl chloride, *p*-toluenesulfonyl
- 20 chloride, even more preferably methanesulfonyl chloride.

The ratio between compound I and sulfonyl halide is typically in the range of 1:2 to 1:10, such as 1:2-1:5, preferably 1:2-1:4, more preferably 1:2.5-1:3.5.

- 25 In one embodiment of the invention, compound I may be treated with two different sulfonyl halides, R^{III}SO₂X and R^{IV}SO₂X, wherein R^{III} and R^{IV} are independently selected from the group of substituents defined for R¹ provided that R^{III} and R^{IV} do not represent the same group, and X is as defined above.
- 30 It should be understood that treatment of compound I with R^{III}SO₂X and R^{IV}SO₂X is performed in two separate steps. First, compound I is treated with R^{III}SO₂X in the ratio 1:1-1:1.5, preferably 1:1-1:1.3, more preferably 1:1.1-1:1.2, to afford compound II, wherein R₄ or R₅ is R^{III}SO₂O- and R₅ or R₄ is hydroxyl. Subsequently, the formed compound II is treated with R^{IV}SO₂X in the ratio 1:1-1:2.5, preferably 1:1-1:2, more preferably 1:1.1-1:1.5

to afford compound II wherein R₄ is R^{III}SO₂O- or R^{IV}SO₂O- and R₅ is R^{IV}SO₂O- if R₄ is R^{III}SO₂O- and R₅ is R^{III}SO₂O- and if R₄ is R^{IV}SO₂O-.

- It should be understood that reaction of compound I with the sulfonyl halide in the
5 presence of an anhydrous base, such as pyridine, 4-dimethylaminopyridine, imidazole,
triethylamine, or sodium hydride, increase the overall yield of the reaction.

In a preferred embodiment of the invention, the treatment is performed in the presence of
pyridine, imidazole, or 4-dimethylaminopyridine, preferably pyridine.

- 10 It should be clear to a person skilled in the art that other sulfonation reagents than sulfonyl
halides can be used in the reaction, such as sulfonic acids and anhydrides.

- For a person skilled in the art, it should also be clear that the treatment of compound I
15 with the sulfonyl halide typically is carried out in the presence of a solvent, such as
pyridine, tetrahydrofuran, toluene, xylene, benzene, ether, ethylacetate, acetonitril,
triethylamine, N,N-dimethylformamide, dimethylsulfoxide, dichloromethane, and 1,2-
dichloroethane.

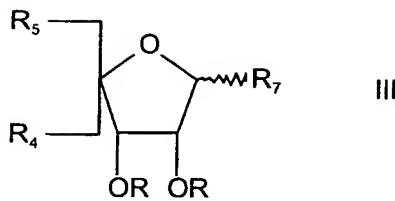
- 20 For a person skilled in the art, it should likewise be clear that the base and the solvent
may be constituted by the same substance, such as pyridine.

The treatment of compound I with sulfonyl halide is typically performed at -70°C to 40°C,
such as -30°C to 40°C.

- 25 In a preferred embodiment of the invention, compound I is treated with sulfonyl halide at
-5°C to 30°C, preferably 0°C to 25°C.

- 30 The present invention also relates to the compound of the general formula II as defined
above.

The present invention furthermore relates to the compound of the general formula III:



wherein R₁, R₄, and R₅ are as defined above; and

- R₆ is selected from hydrogen, optionally substituted (C₁₋₆-alkyl)carbonyl, optionally
5 substituted arylcarbonyl, optionally substituted aryl(C₁₋₆-alkyl), optionally substituted C₁₋₆-
alkyl, and tri-(alkyl/aryl)silyl, such as acetyl, benzoyl, *m*-trifluoromethylbenzoyl, benzyl,
tert-butyldimethylsilyl and *tert*-butyldiphenylsilyl; and

- R₇ is selected from optionally substituted (C₁₋₆-alkyl)carbonyloxy, optionally substituted
10 C₁₋₆-alkoxy, halogen, optionally substituted arylthio, optionally substituted C₁₋₆-alkylthio,
and optionally substituted aryloxy, such as acyloxy, methoxy, ethoxy, chloride, fluoride,
bromide or iodide, or -SC₆H₅.

- In a preferred embodiment of the invention R₁ represents benzyl or 4-phenylbenzyl, most
15 preferably 4-phenylbenzyl, and R₄ and R₅ both are selected from methanesulfonyl,
trifluoromethanesulfonyl, ethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, butanesulfonyl,
nonafluorobutanesulfonyl, α -toluenesulfonyl, *p*-toluenesulfonyl, benzenesulfonyl, *p*-
bromobenzenesulfonyl, and *p*-nitrobenzenesulfonyl, preferably from methanesulfonyl,
trifluoromethanesulfonyl, *p*-toluenesulfonyl and *p*-bromobenzenesulfonyl, more preferably
20 methanesulfonyl, and *p*-toluenesulfonyl, even more preferably methanesulfonyl.

- In a preferred embodiment of the invention R₆ is selected from acetyl, benzoyl and *m*-
trifluoromethylbenzoyl, preferably acetyl, and R₇ is selected from acyloxy, methoxy,
ethoxy, chloride, fluoride, bromide, iodide and -SC₆H₅, preferably acyloxy and methoxy,
25 even more preferably acyloxy.

In the most preferred embodiment of the invention R₁ represents benzyl or 4-
phenylbenzyl, R₄ and R₅ both represent methanesulfonyl, R₆ represents acetyl, and R₇
represents acyloxy.

The key intermediate with the general formula III may be coupled with suitable protected nucleobases resulting in the formation of nucleosides which undergo base-induced ring-closure to afford 2'-O,4'-C-methylene bicyclonucleosides. It should be understood that the formed nucleosides likewise can undergo ring-closure in the presence of different amines, 5 preferably benzylamine, and potassium thioacetate to afford the 2'-N,4'-C-methylene- and 2'-S,4'-C-methylene analogues, respectively.

Compounds with the general formula III may be obtained from compound II by one of the following strategies:

10

treatment of compound II with 80% acetic acid or trifluoroacetic acid followed by treatment of the formed intermediate with acetic anhydride (a corresponding longer chain acid anhydride) in pyridine afford compound III wherein R₆ is acetyl and R₇ is acyloxy;

15

treatment of compound II with HCl in methanol (or a longer chain alcohol) afford compound III wherein R₆ is hydrogen and R₇ is methoxy (or a longer chain alkoxy). The formed compound III can be further transformed to obtain compounds of the formula III where in R₆ is as defined above;

20

treatment of compound II with HCl in methanol afford compound III wherein R₆ is hydrogen and R₇ is methoxy. Transformation of R₆ into one of the groups described above followed by treatment of the formed product with HCl_(g) in dichloromethane afford compound III wherein R₇ is chloro and R₆ is as defined above;

25

conversion of compound II into compound III wherein R₇ is C₆H₅S- is performed as described in the literature.

Synthesis of [2.2.1]bicyclo nucleosides

30 As an illustrative example of synthesis of [2.2.1]bicyclo nucleosides using the method of the present invention (1S, 3R, 4R, 7S)-7-hydroxy-1-hydroxymethyl-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (**7**) was synthesized using 3-O-benzyl-4-C-hydroxymethyl-1,2-O-isopropylidene- α -D-ribofuranose (**1**) as starting material (Figures 1 and 3). Methanesulfonyl chloride (2.7 equivalents) was added to **1** (1 equivalent) in dry pyridine at 35 0°C and the reaction mixture was allowed to heat to room temperature. The reaction

mixture was stirred for 1 hour at room temperature affording the key intermediate **2** in 98% yield after aqueous work up. Compound **2** was used in the following step with out further purification. Subsequent, acetolysis of the intermediate **2** using 80% trifluoroacetic acid followed by acetylation with acetic acid (3 equivalents) in pyridine afforded the key
5 intermediate **3** in 92% yield. Compound **3** was coupled with silylated nucleobase using trimethylsilyl trifluoromethanesulfonate as a Lewis acid according to the methodology developed by Vorbrüggen H (Vorbruggen, K.; Krolikiewicz, K. and Bennua.B., *Chem. Ber.* 114, 1234-1255, (1981). Purification by silica gel flash chromatography afforded the nucleoside **4** in 85% yield. Direct based-induced ring-closure was performed by treating
10 compound **4** with 0.5 M NaOH (1,4-dioxane:H₂O, 1:1) and refluxed overnight. Aqueous work-up and purification by silica gel flash chromatography afforded compound **6** in 88% yield. Catalytic hydrogenation afforded (1S, 3R, 4R, 7S)-7-hydroxy-1-hydroxymethyl-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (**7**) in 84% yield after crystallisation from 10% ethanol in dichloromethane.

15

Synthesis of (1S, 3R, 4R, 7S) -7-hydroxy-1-hydroxymethyl-(guanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane was performed using the same strategy. Guanidine derivatives were also prepared by a similar strategy, such as the (1S,3R,4R,7S)-7-hydroxy-1-hydroxymethyl-3-(2-N-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane **16**, as
20 illustrated by Figure 2.

The advantageous versatility of a strategy according to this invention, wherein the key intermediate (compounds of general formula III) is employed, is further illustrated by the fact that isomers with C2' (nucleoside numbering) inversion are accessible to give α-L-
25 ribose sugars. Thus, the thymidinyl-α-L-ribose **12** was prepared from the key intermediate. This preparation α-L-ribose [2.2.1]bicyclo nucleosides from the key intermediate has been applicable to other naturally occurring and non-naturally occurring nucleobases.

30 The versatility of this route is further illustrated in Figures 4 to 8 wherein [2.2.1]bicyclo nucleoside derivatives of adenosine, cytosine, uridine, thymidine and guanidine are accessible from the key intermediate of the general formula III. Figure 6 illustrates a combination of preferred embodiments for compounds the general formula III for the preparation of [2.2.1]bicyclo nucleoside derivatives of uridine, wherein R⁷ is acetoxy, R⁴

and R⁵ are each mesylate and R¹ is the aryl substituted benzyl, phenylbenzyl (labelled compound **106** in Figure 6).

Figures

5

Figure 1

A general synthetic route is outlined. From the known diol **1**, a critical intermediate **3**, may be conveniently prepared. Using the desired nucleobase or their derivatives (such as thymine, isobutyrylguanidine, N-acetylcytosine, 6-N-benzoyladenine, hypoxantine), 10 ribonucleoside derivatives **4A**, **4B**, **4C**, **4D**, and **4E** can be accessed. Selective protective group manipulation allows for 2,4-cyclisation and access to many LNAs. This scheme is detailed in Examples 1-7.

Figure 2

15 The use of key intermediate **3** to a LNA guanidine derivative **16**. This scheme is detailed in Example 9.

Figure 3

The use of key intermediate **3** to a LNA thymine derivative **12**. This scheme is detailed in 20 Example 8.

Figure 4

The use of key intermediate **3** to a LNA adenine derivative **20**. This scheme is detailed in Example 10.

25

Figure 5

The use of key intermediate **3** to a LNA cytosine derivative **7aC**. This scheme is detailed in Example 12.

30 Figure 6

A modified route in that the bis-isopropylidene **101** is used to access key intermediate **106**, which is of the general formula III. The synthesis sequence comprises: a) N⁴-acetylcytosine, BSA, TMSTf, CH₃CN; b) LiOH, THF/H₂O; c) Sodium benzoate, CsCO₃, DMF; d) Pd(OH)₂, cyclohexane, EtOH; e) i. Bz-Cl, pyridine, ii. NaOH, MeOH, pyridine. A

different protective group strategy is used and the LNA uracil derivatives **110** and **112** are accessed. This scheme is detailed in Example 11.

Figure 7

- 5 Different LNA adenine derivatives are accessible using key intermediate **3**. These offer possible advantages for later oligomerization steps. This scheme is detailed in Example 13. The synthesis sequence comprises: a) NaH, 4-chloromethylbiphenyl, THF/DMF; b) 80% AcOH; c) i. NaIO₄, THF/H₂O; ii. 37% CH₂O, 2M NaOH, dioxane, d) MsCl, pyridine; e) AcOH/Ac₂O/H₂SO₄; f) Uracil, BSA, TMSTf, CH₃CN; g) LiOH, THF/H₂O, h) Sodium 10 benzoate, DMF; i) NH₄OH, MeOH; j) FeCl₃, CH₂Cl₂; k) NH₄OH, MeOH.

Figure 8

The use of the hypoxanthine as nucleobase allows access to the LNA hypoxanthine derivative **25** and the LNA-adenine derivative **27**. This scheme is detailed in Example 14.

15

Definitions

In the present context, the term "C₁₋₆-alkyl" means a linear, cyclic or branched hydrocarbon group having 1 to 6 carbon atoms, such as methyl, ethyl, propyl, iso-propyl, 20 pentyl, cyclopentyl, hexyl, cyclohexyl, preferred examples of "C₁₋₆-alkyl" are methyl, ethyl, propyl, iso-propyl, butyl, *tert*-butyl, iso-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, in particular methyl, ethyl, propyl, iso-propyl, *tert*-butyl, iso-butyl and cyclohexyl.

In the present context, the term "C₃₋₇-alkylene" means a linear biradical having 3 to 7 25 carbon atoms, such as 1,3-propylene, 1,4-butylene, 1,5-pentylene, 1,6-hexylene, and 1,7-heptylene.

In the present context, *i.e.* in connection with the term "alkyl", the term "optionally substituted" means that the group in question may be substituted one or several times, 30 preferably 1-3 times, with group(s) selected from hydroxyl, C₁₋₆-alkoxy, carboxyl, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkylcarbonylamino, cyano, carbamido, halogen, where aryl and heteroaryl may be 35 substituted 1-5 times, preferably 1-3 times, with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino

or halogen. Especially preferred examples are hydroxyl, C₁₋₆-alkoxy, carboxyl, aryl, heteroaryl, amino, mono- and di(C₁₋₆-alkyl)amino, and halogen, where aryl and heteroaryl may be substituted 1-3 times with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino or halogen. Aryl and heteroaryl may be substituted as specifically described below for "optionally substituted aryl and heteroaryl".

In the present context the term "aryl" means a fully or partially aromatic carbocyclic ring or ring system, such as phenyl, naphthyl, 1,2,3,4-tetrahydronaphthyl, anthracyl, phenanthracyl, pyrenyl, benzopyrenyl, fluorenyl and xanthenyl, among which phenyl is a preferred example.

The term "heteroaryl" means a fully or partially aromatic carbocyclic ring or ring system where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen (=N- or -NH), sulphur, and/or oxygen atoms. Examples of such heteroaryl groups are oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl, piperidinyl, coumaryl, furyl, quinolyl, benzothiazolyl, benzotriazolyl, benzodiazolyl, benzooxazolyl, phthalazinyl, phthalanyl, triazolyl, tetrazolyl, isoquinolyl, acridinyl, carbazolyl, dibenzazepinyl, indolyl, benzopyrazolyl, phenoxazonyl.

- 20 In the present context, i.e. in connection with the terms "aryl" and "heteroaryl", the term "optionally substituted" means that the group in question may be substituted one or several times, preferably 1-5 times, in particular 1-3 times with group(s) selected from hydroxyl (which when present in an enol system may be represented in the tautomeric keto form), C₁₋₆-alkyl, C₁₋₆-alkoxy, oxo (which may be represented in the tautomeric enol form), carboxyl, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxy, aryloxy-carbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C₁₋₆-alkyl)amino; carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkylcarbonylamino, cyano, guanidino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, sulphanyl, dihalogen-30 C₁₋₄-alkyl, trihalogen-C₁₋₄-alkyl, halogen, where aryl and heteroaryl representing substituents may be substituted 1-3 times with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino or halogen. Preferred examples are hydroxyl, C₁₋₆-alkyl, C₁₋₆-alkoxy, carboxyl, C₁₋₆-alkoxy-carbonyl, C₁₋₆-alkylcarbonyl, aryl, amino, mono- and di(C₁₋₆-alkyl)amino, and halogen, wherein aryl may be substituted 1-3 times with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino or halogen.

In the present context, the term "tri(alkyl/aryl)silyl" means a silyl group substituted with 0-3 alkyl groups and/or 0-3 aryl groups, with the provision that the total number of alkyl and aryl groups is 3, selected from trimethylsilyl, allyldimethylsilyl, dimethylphenylsilyl,

- 5 diphenylmethylsilyl, isopropyldimethylsilyl, tert-butyldimethylsilyl, and tert-butyldiphenylsilyl,

"Halogen" includes fluoro, chloro, bromo, and iodo.

- 10 In the present context, the term "nucleobase" covers naturally occurring nucleobases as well as non-naturally occurring nucleobases. It should be clear to the person skilled in the art that various nucleobases which previously have been considered "non-naturally occurring" have subsequently been found in nature. Thus, "nucleobase" includes not only the known purine and pyrimidine heterocycles, but also heterocyclic analogues and

- 15 tautomers thereof. Illustrative examples of nucleobases are adenine, guanine, thymine, cytosine, uracil, purine, xanthine, diaminopurine, 8-oxo- N^6 -methyladenine, 7-deazaxanthine, 7-deazaguanine, N^4,N^4 -ethanocytosine, N^6,N^6 -ethano-2,6-diaminopurine, 5-methylcytosine, 5-(C³-C⁶)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridine, isocytosine, isoguanin, inosine and the

- 20 "non-naturally occurring" nucleobases described in Benner et al., U.S. Pat No. 5,432,272. The term "nucleobase" is intended to cover every and all of these examples as well as analogues and tautomers thereof. Especially interesting nucleobases are adenine, guanine, thymine, cytosine, and uracil, which are considered as the naturally occurring nucleobases in relation to therapeutic and diagnostic application in humans.

25

In the present context, the term "nucleoside" means a glycoside of a heterocyclic base. The term "nucleoside" is used broadly as to include non-naturally occurring nucleosides, naturally occurring nucleosides as well as other nucleoside analogues. Illustrative examples of nucleosides are ribonucleosides comprising a ribose moiety as well as

- 30 deoxyribonucleosides comprising a deoxyribose moiety. With respect to the bases of such nucleosides, it should be understood that this may be any of the naturally occurring bases, e.g. adenine, guanine, cytosine, thymine, and uracil, as well as any modified variants thereof or any possible unnatural bases.

EXPERIMENTAL

Example 1

- 5 **3-O-benzyl-4-C-methanesulfonylmethyl-5-methanesulfonyl-1,2-O-isopropylidene- α -D-ribofuranose (2).**
A solution of 3-O-benzyl-4-C-hydroxymethyl-1,2-O-isopropylidene- α -D-ribofuranose (1, 11.1 g, 40 mmol) (Youssefye, R. D.; Verheyden, J. P. H.; Moffatt, J. G., *J. Org. Chem.* 1979, 44, 1301) in dry pyridine (30 mL) was cooled in an ice-bath. Methanesulfonyl chloride (8.3 mL, 108 mmol) was then added under stirring. The mixture was allowed to warm up to room temperature and stirred for 1 hr. Ether (200 mL) was added and the solution was washed with water (3 x 200 mL). Organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to give 16.4 g (98%) of compound (2) as slightly yellow solid.

15

Example 2

- 1,2- di-O-acetyl-3-O-benzyl-4-C-methanesulfonylmethyl-5-O-methanesulfonyl-D-ribofuranose (3).**
20 A solution of compound (2) (16 g, 34 mmol) in 80% trifluoroacetic acid (100 mL) was stirred at room temperature for 1h. The solvents were evaporated to dryness under reduced pressure, the residue was re-dissolved in dichloromethane (200 mL) and washed by saturated aqueous NaHCO₃ (2 x 200 mL). The organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to give colorless oily intermediate. The
25 intermediate was co-evaporated with dry pyridine (2 x 50 mL), dissolved in pyridine and treated by acetic anhydride (12 mL, 103 mmol) overnight. The reaction mixture was quenched by saturated aqueous NaHCO₃ (250 mL) and washed by dichloromethane (2 x 200 mL). Organic layers were combine, dried over Na₂SO₄ and concentrated under reduced pressure to yield compound (3) (15.9 g, 92%) as colorless oily material.

30

Example 3

1-(2-O-acetyl-3-O-benzyl-4-C-methanesulfonyoxymethyl-5-O-methanesulfonyl- β -D-ribofuranosyl)thymine (4a).

- 5 *N*,*O*-bis-(trimethylsilyl)acetamide (4.4 mL, 17.8 mmol) was added to a stirred mixture of
 (3) (2.4 g, 4.7 mmol) and thymine (0.89 g, 7.1 mmol) in dry acetonitrile (200 mL). The
 reaction mixture was refluxed for 1 h before complete dissolution of thymine. Trimethylsilyl
 triflate (1.8 mL, 9.4 mmol) was then added dropwise and refluxing was continued for more
 2 h. The reaction was cooled to room temperature, diluted with dichloromethane (200 mL)
 10 and washed by saturated aqueous solution of sodium hydrogencarbonate (2 x 200 mL).
 The organic layer was dried over Na₂SO₄, concentrated under reduced pressure and
 purified by silica gel flash chromatography using dichloromethane/methanol (98:2 v/v) as
 eluent to yield 2.4 g (85 %) of nucleoside (**4a**) as a white solid material. δ_H (CD₃Cl) 9.33
 (1H, br s, NH), 7.40-7.28 (5H, m, Bn), 7.08 (1H, d, J1.2, 6-H), 5.71 (1H, d, J3.3, 1'-H),
 15 5.58 (1H, dd, J'6.4, J"3.3, 2'-H), 4.70 (1H, d, J6.4, 3'-H), 4.60 (1H, d, J10.8), 4.55 (1H,
 d, J10.8), 4.53 (1H, d, J11.7), 4.38 (1H, d, J10.8), 4.34 (1H, d, J10.8), 4.32 (1H, d, J11.7),
 3.02, 3.00 (2 x 3H, 2 s, methanesulfonyls), 2.11 (3H, s, acetyl), 1.92 (3H, d, J1.1, CH₃). δ_C
 (CD₃Cl) 170.0 (C=O), 163.7 (C-6), 150.1 (C-2), 137.9, 136.6, (C-5, Bn), 128.6, 128.5,
 128.4 (Bn), 111.8 (C-4), 92.4, 84.0, 77.9, 74.8, 73.7, 68.4, 67.5 (ribose, Bn), 37.7, 37.6
 20 (methanesulfonyls), 20.7 (acetyl), 12.6 (CH₃).

Example 4

(1*S*,3*R*,4*R*,7*S*)-7-Benzyl-1-methanesulfonylmethyl-3-(thymine-1-yl)-2,5-

- 25 **dioxabicyclo[2.2.1]heptane (5a)** and
(1*S*,3*R*,4*R*,7*S*)-7-Benzylxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (6a).

To a solution of compound **(4a)** (2 g, 3.48 mmol) in 30 mL of 1,4-dioxane were added 1 M aqueous NaOH (30 mL) and the mixture was stirred for 10 min at room temperature. TLC 30 analysis (silica gel, 5% methanol/dichloromethane) shown quantitative conversion of starting material into a intermediate with a slightly low mobility. Analytical amount of the reaction mixture was divided by extraction in system dichloromethane/saturated aqueous NaHCO₃. Organic layer was washed by water, dried over Na₂SO₄ and concentrated under reduced pressure to give compound **(5a)** as a white solid material. δ_H (CD₃Cl) 9.24 (1H, br s, NH), 7.41-7.22 (6H, m, 6-H, Bn), 5.68 (1H, s, 1'-H), 4.66 (1H, d, J, 11.5, Bn), 4.61 (1H,

s, 2'-H), 4.59 (1H, d, *J*12.1, 5'-H), 4.56 (1H, d, *J*11.5, Bn), 4.52 (1H, d, *J*12.1, 5'-H,), 4.08 (1H, d, *J*7.9, 1''-H), 3.93 (1H, s, 3'-H), 3.87 (1H, d, *J*7.9, 1''-H), 3.08 (3H, s, methanesulfonyl), 1.93 (3H, s, CH₃). δ_H (CD₃Cl) 163.6 (C-6), 149.6 (C-2), 136.3, 134.0 (C-5, Bn), 128.4, 128.2, 127.8 (Bn), 110.7 (C-4), 87.5, 85.5, 76.6, 75.9, 72.3, 71.5, 64.0
5 (ribose, Bn), 37.8 (methanesulfonyl), 12.4 (CH₃).

The reaction mixture was then refluxed overnight, diluted with 200 mL of dichloromethane and washed by saturated aqueous NaHCO₃ (2 x 200 mL). Organic phase was dried, solvents were removed under reduced pressure and the residue was purified by silica gel
10 flash chromatography using 3% methanol/dichloromethane as eluent. Compound (**6a**) (1.1 g, 88 %) was obtained after removing of solvent as a white solid material. δ_H (CD₃Cl) 9.28 (1H, br s, NH), 7.45 (1H, d, *J*1.1, 6-H), 7.38-7.22 (5H, m, Bn), 5.66 (1H, s, 1'-H), 4.67 (1H, d, *J*11.6, Bn), 4.56 (1H, d, *J*11.7, Bn), 4.54 (1H, s, 2'-H), 4.05 (1H, d, *J*7.9, 1''-H), 4.01 (1H, d, *J*12.5, 5'-H), 3.96 (1H, s, 3'-H), 3.95 (1H, d, *J*12.6, 5'-H), 3.83 (1H, d, *J*7.9, 1''-H), 1.88 (3H, d, *J*1.1, CH₃). δ_C (CD₃Cl) 163.9 (C-6), 149.8 (C-2), 137.0, 134.7 (C-5, Bn), 128.5, 128.2, 127.8 (Bn), 110.3 (C-4), 88.2, 87.3, 76.9, 75.9, 72.3, 72.0, 57.6 (ribose, Bn), 12.7 (CH₃).

Example 5

20 (1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (**7a**).
A mixture of compound (**6a**) (3 g, 8.4 mmol), 20% Pd(OH)₂ /C (1.5 g) and ammonium formate (1.6 g) was suspended in 20 mL of methanol. After refluxing the mixture for 10
25 min, the catalyst was filtered off through celite column and washed by methanol. All the filtrate was combined and concentrated to give a white solid material. The latter was crystallized from 10 % ethanol/dichloromethane to yield 1.9 g (84 %) of compound (**7a**) which had the same chromatographic mobility (silica TLC) and H¹- and C¹³-NMR spectra as authentic compound (Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar,
30 R.; Meldgaard, M.; Olsen, C. E.; Wengel, J., *Tetrahedron* **1998**, *54*(14), 3607).

Example 6**9-(2-O-acetyl-3-O-benzyl-4-C-methanesulfoxymethyl-5-O-methanesulfonyl- β -D-ribofuranosyl)-2-N-isobutyrylguanine (4b).**

- 5 To a stirred suspension of the anomeric mixture (**3**) (2.3 g, 4.6 mmol) and 2-N-isobutyrylguanine (1.8 g, 7.9 mmol) in anhydrous 1,2-dichloroethane (150 mL) was added *N,O*-bis(trimethylsilyl)acetamide (5 mL, 20.4 mmol). The mixture was refluxed for 1 h before complete dissolution of 2-N-isobutyrylguanine. Trimethylsilyl triflate (2 mL, 11.0 mmol) was then added and the solution was stirred at reflux for more 2 h. The reaction
- 10 mixture was allowed to cool to room temperature, diluted by dichloromethane (200 mL) and washed with saturated aqueous solution of sodium hydrogencarbonate (2 x 200 mL). The solvents were removed under reduced pressure and the residue was purified by silica gel flash chromatography in gradient concentration (1-2 %) methanol/dichloromethane as eluent to give 2.1 g (68%) of white solid material consisted of three isomers (compound
- 15 (**4b**) ca. 90 % purity).

An analytical amount of (**4b**) was additionally purified by re-chromatography at the same conditions. δ_H (CD₃Cl) 12.22 (1H, br s, NHCO), 9.34 (1H, br s, NH), 7.76 (1H, s, 8-H), 7.40-7.30 (5H, m, Bn), 6.03 (1H, d, J3.9, 1'-H), 5.76 (1H, dd, J'6.0, J''3.9, 2''-H), 5.08 (1H, d, J6.0, 3''-H), 4.91 (1H, d, J10.5), 4.67 (1H, d, J10.9), 4.61 (2H, d, J11.1), 4.49 (1H, d, J10.5), 4.39 (1H, d, J11.0), 4.32 (1H, d, J11.7), 3.14, 3.02, (2 x 3H, 2 s, methansulfonyls), 2.70 (1H, m, CHCO), 2.09 (3H, s, acetyl) 1.24 (6H, m, CH₃CH). δ_C (CD₃Cl) 179.3 (COCH), 169.8 (COCH₃), 155.0, 148.1, 147.1 (guanine), 138.9, 136.6 (guanine, Bn), 128.6, 128.4, 128.2 (Bn), 122.2 (guanine), 88.6, 84.4, 78.2, 74.8, 74.3, 67.9, 67.4 (ribose, Bn), 37.8, 37.7, (methanesulfonyls), 36.3 (COCH), 20.6 (COCH₃), 19.0, 18.9 (CH₃CH).

Example 7

- 30 (**1S,3R,4R,7S**)-7-Benzylxy-1-methanesulfoxymethyl-3-(guanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (**5b**) (**1S,3R,4R,7S**)-7-Benzylxy-1-hydroxymethyl-3-(guanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (**6b**) and (**1S,3R,4R,7S**)-7-Hydroxy-1-hydroxymethyl-3-(guanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (**7a**). Nucleoside (**4b**) was dissolved in 0.5 M aqueous sodium hydroxide and kept at ambient
- 35 temperature. The reaction was followed by RP-HPLC analysis in system A:

Column: Delta-Pack, C18, 100Å, 3.9 x 150 mm.

Gradient: 0 to 50 % acetonitrile in water (0.05 M triethylammonium acetate, pH 7.0) during 15 min.

Flow rate: 1.5 mL/min.

- 5 Starting material (**4b**) (retention time 19.5 min) was fully consumed during 1h at ambient temperature to give a number of intermediate products. The main product had retention time of 17.9 min and was assumed to be the 2-N-isobutyryl protected derivative of nucleoside **5b**. The complete removal of isobutyryl group was observed after 12 h of reaction (**5b**; retention time 14.7 min; ca.90 % purity by HPLC analysis). Only trace 10 amounts of nucleoside (**6b**) have been found in the reaction mixture. The reaction proceed at reflux for more 12 h which resulted in full conversion of (**5b**) to (**6b**) (retention time 12.6 min). The reaction mixture was neutralized by acetic acid (to pH 7), filtered through silica gel column and concentrated under reduced pressure. Analytical amount of compound (**6b**) was purified by semi-prep RP-HPLC (Nucleosil C18, 10 x 30 mm) 15 using the same solvents as in system A. Compound (**6b**) (ca.10 mg) was dissolved in methanol, 10 % Pd/C (50 mg) and ammonium format (20 mg) were added and the mixture was refluxed for 15 min. Analysis of the reaction mixture in system A shown a quantitative formation of compound (**7b**) (retention time 4.7 min) with the same mobility as authentic compound prepared by method described earlier (Koshkin, A. A.; Singh, S. 20 K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J., *Tetrahedron* **1998**, *54*(14), 3607).

Example 8

- 25 **1-(3-O-benzyl-4-C-methanesulfonylmethyl-5-O-methanesulfonyl-β-D-ribofuranosyl)thymine (8).**
To a solution of compound **4a** (2.8 g, 4.8 mmol) in 1,4-dioxane (10 mL) was added concentrated ammonium hydroxide solution (30 %, 1 mL). After 4 h, the solvents were removed under reduced pressure, the residue re-dissolved in dichloromethane and 30 applied for silica gel HPLC using 0 to 3 % methanol/dichloromethane mixture as eluent to yield 2.05 g (78 %) of compound **8** as a white solid material.

1-(3-O-benzyl-4-C-methanesulfonylmethyl-2,5-di-O-methanesulfonyl- β -D-ribofuranosyl)thymine (9).

Compound **8** (2 g, 3.7 mmol) was co-evaporated with anhydrous pyridine (2 x 50 mL), dissolved in pyridine (50 mL) and reacted with methanesulfonyl chloride (0.35 mL, 4.5 mmol) overnight. The mixture was diluted with dichloromethane (100 mL), washed with saturated aqueous NaHCO₃ (2x 100 mL) and concentrated under reduced pressure. Column silica gel chromatography (2 % methanol/dichloromethane as eluent) yielded compound **9** (2.1 g, 92 %) as white solid material. δ_H (CDCl₃) 9.67 (1H, s, NH), 7.38-7.15 (6H, m, 6-H, Bn), 5.81 (1H, d, J 2.4, 1'-H), 5.58 (1H, dd, J'6.5, J''2.4, 2'-H), 4.75 (1H, d, J 11.0), 4.73 (1H, d, J 6.6, 3'-H), 4.60 (1H, d, J 10.8), 4.53 (1H, d, J 11.5), 4.41 (1H, d, J 11.0), 4.35 (1H, d, J 11.0), 4.33 (1H, d, J 11.6), 3.20, 3.12, 3.00 (3 x 3H, 3 s, methanesulfonyls), 1.91 (3H, d, J 1.1, 5-CH₃). δ_C (CDCl₃) 163.7 (C-6), 150.3 (C-2), 137.8, 136.2 (C-5, Bn), 128.6, 128.5, 128.4, 128.3 (Bn), 111.7 (C-4), 93.1, 84.2, 77.6, 76.8, 74.1, 68.1, 67.5, (ribose, Bn), 38.5, 37.5, 37.4 (methanesulfonyls), 12.1 (5-CH₃).

15

(1*R*,3*R*,4*S*,7*S*)-7-Benzyl-1-methanesulfonylmethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (10).

Compound **9** (105 mg, 0.17 mmol) was dissolved in a mixture of dioxane and water (2:1, 15 mL). 2 M aqueous solution of NaOH was added by portions of 100 uL every 0.5 hrs 20 and the reaction was followed by analytical TLC (silica gel, 5 % methanol/dichloromethane). Two intermediates with lower mobility were detected in the reaction mixture which were completely converted to a single product after addition of 1 mL of aqueous NaOH solution. The product was extracted by dichloromethane (50 mL), washed with saturated aqueous NaHCO₃ (50 mL) and brine (40 mL), and dried over 25 Na₂SO₄. Concentration under reduced pressure gave 72 mg (96 %) of compound **10** as a white solid material. δ_H (CDCl₃) 8.90 (1H, br s, NH), 7.48-7.34 (6H, m, 6-H, Bn), 6.27 (1H, s, 1'-H), 4.72 (1H, d, J 11.7), 4.66 (1H, d, J 11.7), 4.56 (1H, d, J 11.7), 4.48 (1H, d, J 11.7), 4.48 (1H, dd, J'2.4, J''1.1, 2'-H), 4.25 (1H, d, J 2.4, 3'-H), 4.10 (1H, d, J 9.1), 4.05 (1H, d, J 9.0), 3.05, (3H, s, methanesulfonyl), 1.95 (3H, d, J 1.1, 5-CH₃). δ_C (CDCl₃) 163.5 (C-6), 150.1 (C-2), 136.2, 135.5 (C-5, Bn), 128.7, 128.6, 128.1, (Bn), 109.9 (C-4), 90.0, 86.0, 81.7, 75.9, 73.2, 67.0, 65.1 (ribose, Bn), 37.5, (methanesulfonyl), 12.6 (5-CH₃).

(1*R*,3*R*,4*S*,7*S*)-7-Benzylxyloxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (11).

Compound 9 (1.8 g, 2.94 mmol) was suspended in 0.5 M solution of aqueous NaOH (1,4-dioxane/water 1/1, 80 mL) and the mixture was heated at 90°C for 48 hrs. The solution
5 was cooled to room temperature, diluted with dichloromethane (100 mL) and washed with saturated aqueous NaHCO₃ (2 x 100 mL). The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The final product (compound 11, 800 mg, 76 %) was purified by silica gel column chromatography using 1 to 4 % solution of ethanol/dichloromethane as eluent. δ_H (CDCl₃) 9.38 (1H, br s, NH), 7.52 (1H, d, J 1.1, 6-H), 7.40-7.31 (5H, m, Bn),
10 6.24 (1H, s, 1'-H), 4.72 (1H, d, J 11.9), 4.65 (1H, d, J 11.9), 4.48 (1H, dd, J 2.2, J'' 0.8, 2'-H), 4.22 (1H, d, J 2.3, 3'-H), 4.08 (1H, d, J 9.7), 4.05 (1H, d, J 12.3), 4.02 (1H, d, J 9.8),
3.91 (1H, d, J 12.2), 1.92 (3H, d, J 1.1, 5-CH₃). δ_C (CDCl₃) 164.0 (C-6), 150.3 (C-2), 136.6,
135.9 (C-5, Bn), 128.6, 128.3, 127.8 (Bn), 109.5 (C-4), 89.8, 88.5, 81.8, 76.0, 73.8, 72.9,
59.0 (ribose, Bn), 12.5 (5-CH₃).

15

(1*R*,3*R*,4*S*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (12).

A mixture of compound 11 (750 mg, 2.09 mmol) and 10% Pd/C (500 mg) was suspended in methanol (20 mL) and sodium formate (700 mg, 11.1 mmol) was added. The reaction
20 was conducted at refluxing for 10 min and cooled to ambient temperature. The catalyst was filtered off and the mixture was concentrated under reduced pressure to give compound 12 (540 mg, 96 %) as a white solid material.
δ_H (DMSO-d₆) 11.32 (1H, br s, NH), 7.64 (1H, s, 6-H), 6.09 (1H, s, 1'-H), 5.91 (1H, d, J 3.1, 3'-OH), 4.94 (1H, br s, 5'-OH), 4.31, 4.17 (2 x s, 2'-H, 3'-H), 4.03 (2H, s, 1''-H), 3.71
25 (2H, s, 5'-H), 1.82 (3H, s, 5-CH₃). δ_C (DMSO-d₆) 163.9 (C-6), 150.4 (C-2), 136.5 (C-5),
108.0 (C-4), 89.2, 89.1, 77.3, 74.7, 73.6, 57.2 (ribose), 12.3 (5-CH₃).

Example 9

30 **(1*S*,3*R*,4*R*,7*S*)-7-Benzylxyloxy-1-methanesulfonyloxymethyl-3-(2-N-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (13).**

Compound 4b (1g, 1.49 mmol) was dissolved in an aqueous 0.5 M NaOH/dioxane mixture (1:1, 20 mL) and the solution was kept at room temperature for 15 min. Dichloromethane (20 mL) was added and the mixture was washed with saturated NaHCO₃ (2 x 30 mL).
35 After separation organic layer was dried over Na₂SO₄, concentrated under reduced

pressure, and the residue was purified by column silica gel chromatography using 0 to 4 % methanol/dichloromethane as eluent to yield 620 mg (78%) of compound 13 as a white solid material.

δ_H ($CDCl_3$) 12.14 (1H, br s, NHCO), 9.51 (1H, br s, NH), 7.77 (1H, s, 8-H), 7.30-7.26 (5H, m, Bn), 5.84 (1H, s, 1'-H), 4.67 (1H, d, J 11.5), 4.63 (1H, d, J 12.0), 4.62 (1H, s, 2'-H), 4.62 (1H, d, J 11.5), 4.56 (1H, d, J 11.9), 4.50 (1H, s, 3'-H), 4.12 (1H, d, J 8.0, 1''-H), 3.93 (1H, d, J 7.9, 1''-H), 3.06 (3H, s, methanesulfonyl), 2.77 (1H, m, CHCO), 1.26 (6H, m, CH_3).

10 **(1S,3R,4R,7S)-7-Benzylxyloxy-1-benzoyloxymethyl-3-(2-N-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (14).**

A mixture of compound 13 (600 mg, 1.26 mmol) and sodium benzoate (310 mg, 2.16 mmol) was suspended in anhydrous DMF (25 mL) and heated at 100°C for 4 h under intensive stirring. The solution was cooled to ambient temperature, diluted with 15 dichloromethane (50 mL) and filtered through glass filter. The filtrate was washed with saturated aqueous solution of $NaHCO_3$ (2 x 50 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The final product was purified by silica gel column chromatography (1 to 2.5 % methanol/dichloromethane as eluent) to yield 560 mg (89%) of compound 14 as a white solid material. δ_H ($CDCl_3$) 12.12 (1H, br s, NHCO), 9.30 (1H, br s, NH), 7.92 (m, 2H, Bz), 7.72 (1H, s, 8-H), 7.57 (1H, m, Bz), 7.42 (2H, m, Bz), 7.24-7.20 (5H, m, Bn), 5.81 (1H, s, 1'-H), 4.80 (1H, d, J 12.6), 4.66 (1H, s, 2'-H), 4.64 (1H, d, J 12.0), 4.61 (1H, d, J 12.7), 4.21 (1H, d, J 8.1, 1''-H), 4.20 (1H, s, 3'-H), 4.00 (1H, d, J 7.9, 1''-H), 2.77 (1H, m, CHCO), 1.27 (6H, m, CH_3). δ_C ($CDCl_3$) 178.8 (CHCO), 165.7 (Bz), 154.9, 147.8, 146.9 (guanine), 136.4, 135.3, 133.4 (guanine, Bn, Bz), 129.3, 129.0, 128.6, 25 128.5, 128.2, 128.7 (Bn, Bz), 121.0 (guanine), 86.2, 85.5, 77.1, 72.4, 72.1, 59.3 (ribose, Bn), 36.2 (CHCO), 18.8 (CH_3CH).

(1S,3R,4R,7S)-7-Benzylxyloxy-1-hydroxymethyl-3-(2-N-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (15).

30 To a solution of compound 14 (8.2 g, 14.7 mmol) in ethanol/pyridine (8:1, 450 mL) was added 2 M aqueous solution of NaOH (15.5 mL) and the mixture was stirred for 30 min at ambient temperature. Acetic acid (25 mL) was added to the reaction mixture and the solvents were removed under reduced pressure. The residue was crystallised from 20 % aqueous ethanol to give 5.8 g (87 %) of compound 15 as a white solid material. δ_H (DMSO- d_6) 8.05 (1H, s, 8-H), 7.33-7.26 (5H, m, Bn), 5.85 (1H, s, 1'-H), 5.17 (1H, t, J 5.4,

5'-OH), 4.69 (1H, s, 2'-H), 4.64 (2H, s, Bn), 4.23 (1H, s, 3'-H), 3.95 (1H, d, *J* 7.9, 1''-H), 3.83 (2H, m, 5'-H), 3.80 (1H, d, *J* 8.0, 1''-H), 2.78 (1H, m, CHCO), 1.12 (6H, m, CH₃). δ_c (CDCl₃) 180.2 (CHCO), 154.8, 148.2, 147.7 (guanine), 137.9, 136.3, (guanine, Bn), 128.3, 127.6, 127.5 (Bn), 120.5 (guanine), 88.2, 85.2, 76.9, 72.1, 71.3, 56.7 (ribose, Bn), 34.8 5 (CHCO), 18.9 (CH₃CH).

(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (16).

To a solution of compound 15 (5.8 g, 12.7 mmol) in methanol (50 mL) was added 10 % 10 Pd/C (2 g) and formic acid (3 mL). The mixture was refluxed for 5 h, cooled to ambient temperature and filtrated through silica gel column. The column was washed with methanol (50 mL), all the filtrate was concentrated under reduced pressure to yield 4.55 g (98 %) of compound 16 as a glass-like solid.

15 **Example 10**

1-(2-O-acetyl-3-O-benzyl-4-C-methanesulfonylmethyl-5-O-methanesulfonyl- β -D-ribofuranosyl)-6-N-benzoyladenine (4D).

To a suspension of compound 3 (4.8 g, 9.4 mmol) and 6-N-benzoyladenine (2.7 g, 11.3 20 mmol) in anhydrous 1,2-dichloroethane (40 mL) was added BSA (5.9 mL, 23.8 mmol) and the mixture was refluxed for 1 h. Then trimethylsilyl triflate (2.6 mL, 14.3 mmol) was added, the reaction was refluxed for more 4 h and kept at room temperature overnight. The reaction mixture was diluted with dichloromethane (100 mL), washed with saturated aqueous NaHCO₃ (200 mL), concentrated under reduced pressure, and the product was 25 purified by column silica gel chromatography (1 to 3 % of methanol/dichloromethane as eluent). Yield 3.5 g (53 %) of compound 17 as yellow foam. δ_H (CD₃Cl) 8.76 (1H, s, 8-H), 8.12 (1H, s, 2-H), 8.02 (2H, m, Bz), 7.61 (1H, m, Bz), 7.51 (2H, m, Bz), 7.40-7.34 (5H, m, Bn), 6.23 (1H, d, *J* 3.5, 1'-H), 6.08 (1H, dd, *J'* 5.9, *J''* 3.5, 2'-H), 5.12 (1H, d, *J* 6.0, 3'-H), 4.68 (1H, d, *J* 11.1), 4.67 (1H, d, *J* 11.6), 4.64 (1H, d, *J* 11.0), 4.44 (1H, d, *J* 10.8), 4.39 30 (1H, d, *J* 11.7), 4.36 (1H, d, *J* 11.0), 3.03, 2.87 (2 x 3H, 2 s, methanesulfonyls), 2.13 (3H, s, acetyl). δ_c (CD₃Cl) 169.5 (CH₃CO), 164.5 (Bz), 152.5, 150.9, 149.7, 142.4 (adeninyl), 136.3, 133.2, 132.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8 (Bn, Bz), 123.5 (adeninyl), 87.8, 84.1, 77.3, 74.6, 73.4, 67.3, 67.2 (ribose, Bn), 37.6, 37.3 (methanesulfonyls), 20.5 (acetyl).

(1*S*,3*R*,4*R*,7*S*)-7-Benzylxyloxy-1-methanesulfonoxymethyl-3-(adenin-9-yl)-2,5-dioxabicyclo-[2.2.1]heptane (18).

To a solution of compound **17** (2.5 g, 3.6 mmol) in 1,4-dioxane (20 mL) was added concentrated ammonium hydroxide (30 %, 20 mL). The solution was kept at room

- 5 temperature overnight and diluted with aqueous NaOH (2 M, 5 mL). 30 Min later the solvents were removed under reduced pressure and the residue was suspended in dichloromethane (100 mL), washed with saturated NaHCO₃ (100 mL), dried over Na₂SO₄, and concentrated to a solid foam. Finally, the product was purified by column silica gel chromatography using 2 to 5 % of methanol in dichloromethane as eluent to yield 1.26 g
10 (78 %) of compound **18** as a yellow solid material. δ_H (CD₃Cl) 8.30 (1H, s, 8-H), 7.90 (1H, s, 2-H), 7.31-7.27 (5H, m, Bn), 6.04 (1H, s, 1'-H), 4.93 (1H, s, 2'-H), 4.68 (1H, d, J 11.7), 4.60 (1H, d, J 11.7), 4.59 (1H, d, J 11.7), 4.57 (1H, d, J 11.9), 4.35 (1H, s, 3'-H), 4.19 (1H, d, J 7.9, 1"-H), 4.02 (1H, d, J 7.9, 1"-H), 3.03 (3H, s, methanesulfonyl). δ_C (CD₃Cl) 155.4 (C-6), 152.9 (C-2), 148.6 (C-4), 138.0 (C-8), 136.4, 128.4, 128.2, 127.8 (Bn), 119.7 (C-5),
15 86.6, 85.1, 77.5, 76.8, 72.4, 72.2, 64.4 (ribose, Bn), 37.7 (methanesulfonyl).

(1*S*,3*R*,4*R*,7*S*)-7-Benzoyloxy-1-benzoyloxymethyl-3-(adenin-9-yl)-2,5-dioxabicyclo-[2.2.1]heptane (19).

Sodium benzoate (0.77 g, 5.38 mmol) was added to a solution of compound **18** (1.2 g,

- 20 2.69 mmol) in anhydrous DMF (50 mL). The mixture was stirred at 80°C overnight, cooled to room temperature and filtered through a glass filter. The filtrate was diluted with dichloromethane (100 mL), washed with saturated aqueous NaHCO₃, dried over Na₂SO₄, and concentrated under reduced pressure. The desired compound was purified by silica gel chromatography (1.5 to 4 % methanol in dichloromethane) and crystallised from
25 ethanol to yield 1.04 g (82 %) of compound **19** as a white solid material. δ_H (DMSO/methanol 1/10) 8.16 (1H, s, 8-H), 8.03 (1H, s, 2-H), 8.02 (2H, m, Bz), 7.63 (1H, m, Bz), 7.47 (2H, m, Bz), 7.29-7.18 (5H, m, Bn), 6.07 (1H, s, 1'-H), 4.87 (1H, s, 2'-H), 4.83 (1H, d, J 12.8), 4.71 (1H, d, J 11.9), 4.70 (1H, d, J 12.8), 4.62 (1H, d, J 11.9), 4.47 (1H, s, 3'-H), 4.23 (1H, d, J 8.0, 1"-H), 4.05 (1H, d, J 7.9, 1"-H).

30

(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(6-N-benzoyladenin-9-yl)-2,5-dioxabicyclo-[2.2.1]heptane (20).

A mixture of compound **19** (0.95 g, 2.01 mmol) and Pd(OH)₂/C (20 %, 1 g) was suspended in methanol/cyclohexene (1:1, 20 mL) and refluxed overnight. The reaction

- 35 mixture was cooled to rt, filtered through Celite™ column, and concentrated under

reduced pressure. The residue was co-evaporated with anhydrous pyridine (2 x 20 mL), dissolved in anhydrous pyridine, and cooled in ice-bath. Benzoyl chloride (1.15 mL, 10 mmol) was added dropwise and the mixture was stirred at RT for 20 h. Reaction was then quenched by addition of ice-cold water (40 mL), and washed with dichloromethane (2 x 50 mL). The organic layers were combined, concentrated under reduced pressure, re-dissolved in pyridine/methanol (1:2, 30 mL), and 2 M aqueous NaOH (5 mL) was added. After 15 min, the mixture was neutralised with acetic acid (5 mL) and solvents were removed to give an oily residue. The latter was suspended in 5 % methanol/dichloromethane, applied to a silica gel column and eluted by 5 to 15 % of methanol/dichloromethane as a solvent. The fractions containing compound **20** were concentrated to yield 0.54 g (70 %) of glass-like solid material with the same chromatographic mobility as authentic compound.

Example 11

15

Preparation of diol **104**

Sodium hydride (1.15 g of a 60 % dispersion in mineral oil, 28.75 mmol) was suspended in dry DMF (10 mL) under N₂ and cooled in an ice bath. A mixture of 1,2:5,6-di-O-isopropylidene- α -D-allofuranose **101** (5.0 g 19.21 mmol) and 4-(chloromethyl)-biphenyl (4.67 g, 23.04 mmol, Fluka, >97 %) in dry THF (50 mL) was added dropwise over 45 min. The cooling bath was removed and the mixture was stirred at room temperature for 24 h. The brownish mixture was cooled in an ice bath and water (20 mL) was carefully added. Layers were separated and the aqueous layer was extracted with ether (50 mL). The combined organic layers were washed with water (2 x 50 mL) and brine (50 mL), dried (Na₂SO₄) and evaporated under reduced pressure. To the resulting brown oil, which crystallised on standing, was added 80% acetic acid (40 mL) and the reaction mixture was stirred at room temperature for 24 hours. The mixture was extracted with light petroleum ether (2 x 25 mL) and the acetic acid was evaporated under reduced pressure followed by co-evaporation with ethanol. The residue was partitioned between CH₂Cl₂ (100 mL) and saturated aqueous NaHCO₃ (50 mL). Layers were separated and the aqueous layer was extracted with CH₂Cl₂ (100 mL). The combined organic layers were washed with brine (50 mL), dried (Na₂SO₄) and evaporated under reduced, affording a sticky pale yellow foam (6.9 g). This crude 5,6-diol product **103** (6.9 g) was dissolved in THF/H₂O (50 % v/v, 100 mL) and NaIO₄ (4.6 g, 21.51 mmol) was added. The

reaction mixture was stirred at room temperature for 60 min. and the resulting thick white slurry was filtrated. The formed precipitate was washed with ether (100 mL) and the combined filtrates were extracted with ether (2 x 100 mL). The combined organic layers were washed with water (2 x 100 mL) and brine (100 mL). The solvents were removed under reduced pressure and *p*-dioxane (40 mL) was added to this crude aldehyde product. To the stirred solution was added 37% aqueous formaldehyde (4.0 mL) followed by addition of 2 M aqueous NaOH (18 mL) and the reaction mixture was stirred at room temperature for 21 hours. Saturated aqueous NaHCO₃ (100 mL) was added and the mixture was extracted with CH₂Cl₂ (3 x 200 mL). The combined organic layers were washed with saturated aqueous NaHCO₃ (100 mL), dried (Na₂SO₄) and evaporated under reduced pressure. The residual pale yellow solid material was recrystallised from ether and gave diol 104 as a white solid material (3.7 g). The remaining material in the mother liqueur was not further purified for the time being.¹H NMR (400 MHz, CDCl₃) δ: 1.34 (3H, s, CH₃), 1.65 (3H, s, CH₃) 5 3.61 (1H, "d", J 12.08, H-1''a) 3.81 (1H, d, J 12.10, H-1''b), 3.91 (1H, d, J 10.78, H-5'a) 3.96 (1H, d, J 10.78, H-5'b), 4.26 (1H, d, J 5.31, H-3'), 4.61 (1H, d, J 11.72, phenylbenzyl-CHa), 4.68 (1H, dd, J 4.03 and 5.13, H-2'), 4.84 (1H, d, J 11.72, phenylbenzyl-CHb), 5.78 (1H, d, J 3.84, H-1'), 7.36-7.58 (5H, m, Ar), 7.59- 10 7.60 (4H, m, Ar). ¹³C NMR (400 MHz, CDCl₃) δ: 25.78, 26.45 (C(CH₃)₂, isopropylidene), 63.13, 64.14, 72.30, 77.22, 78.31 (C-1'', C-5', CH₂-phenylbenzyl, C-3', C-2'), 86.16 (C-4'), 104.32 (C-1'), 113.40 (C(CH₃)₂), 126.96, 127.17, 127.29, 128.14, 128.66, 136.08, 140.53, 141.00 (Ar).

Preparation of bis-mesylate 105

To a stirred solution of diol 104 (3.69 g, 9.55 mmol) in dry pyridine (25 mL) under N₂ at 0 - 5°C was added methanesulfonyl chloride (1.86 mL, 24.03 mmol) dropwise. The cooling bath was removed and the reaction mixture was stirred at room temperature for 2.5 hours. The mixture was diluted with ether (100 mL) and washed with saturated aqueous NaHCO₃ (2 x 30 mL), 1 M NaOH (2 x 30 mL), water (30 mL) 15 and brine (30 mL). The organic solution was dried (MgSO₄) and the solvents were evaporated under reduced pressure. Residual pyridine was removed by co-evaporation with toluene and drying under high vacuum over night. The crude bis-mesylate product 5 (4.75 g, 92 % yield, yellow foam) was used without further purification.

Preparation of bis-acetyl 106

Crude bis-mesylate **105** (4.75 g, 8.75 mmol) was dissolved in a mixture of acetic acid (70 mL) and acetic anhydride (7.0 mL) under N₂. To the stirred mixture was 5 added concentrated H₂SO₄ (0.07 mL) and the resulting reaction mixture was stirred at room temperature for 3 hours. The mixture was poured into water (150 mL) containing some ice and stirred for 20 min. Then saturated aqueous NaHCO₃ (100 mL) and CH₂Cl₂ (200 mL) was added and the mixture stirred for 30 min. Layers were separated and the organic layer was washed with saturated aqueous NaHCO₃ (2 x 10 100 mL), dried (Na₂SO₄) and evaporated under reduced pressure. The remaining brown oil was purified by column chromatography on silica: packed in CH₂Cl₂; elution with 0 - 2 % MeOH in CH₂Cl₂, v/v and gave bis-acetylated compound **106** (3.91 g, 76 % yield) as a white foam.

15 Preparation of nucleoside 107

Anomeric mixture **106** (3.91 g, 6.65 mmol) was dissolved in dry CH₃CN (40 mL) under N₂. Uracil (894 mg, 7.98 mmol) was added followed by dropwise addition of *N,O*-bis(trimethylsilyl)acetamide (8.3 mL, 33.58 mmol). The slightly turbid solution was heated to 40°C and stirred at this temperature for 40 min. The clear yellow 20 solution was cooled to room temperature and trimethylsilyl triflate (1.54 mL, 7.97 mmol) was added dropwise. The reaction mixture was heated to reflux and stirred at this temperature for 5 hours. The mixture was cooled to room temperature and stirred over night. The mixture was diluted with CH₂Cl₂ (150 mL) and washed with saturated aqueous NaHCO₃ (3 x 50 mL), dried (Na₂SO₄) and evaporated under 25 reduced pressure. The residue was subjected to column chromatography on silica: packed in CH₂Cl₂, elution with 1 - 2 % MeOH in CH₂Cl₂, v/v, and gave the coupled product **107** (2.99 g 70 % yield) as a pale yellow foam.

Preparation of cyclised nucleoside 108

30 To a solution of nucleoside **107** (2.9 g, 4.50 mmol) in THF (25 mL) and water (20 mL) was added lithium hydroxide monohydrate (953 mg, 22.70 mmol) and the reaction mixture was stirred at room temperature for 2 hours. The organic solvent was evaporated under reduced pressure and the residue was diluted with CH₂Cl₂ (150 mL), washed with saturated aqueous NaHCO₃ (2 x 50 mL), dried (Na₂SO₄) and

evaporated under reduced pressure. The remaining yellow foam was purified by column chromatography on silica: packed in CH_2Cl_2 , elution with 0 - 1 % MeOH in CH_2Cl_2 , v/v, affording the cyclised product 108 (1.64 g, 73 % yield) as an off-white foam.

- 5 ^1H NMR (400 MHz, CDCl_3) δ : 3.06 (3H, s, CH_3), 3.91 (1H, d, J 7.87, H-1''a), 3.94 (1H, s, H-3'), 4.12 (1H, d, J 8.06, H-1''b), 4.58 (2H, s, CH_2), 4.59 (1H, d, J 12.81, H-5'a), 4.67 (1H, s, H-2'), 4.70 (1H, d, J 11.53, H-5'b), 5.67 (1H, s, H-1'), 5.75 (1H, d, J 8.24, H-5), 7.33-7.45 (5H, m, phenylbenzyl), 7.56-7.59 (5H, m, phenylbenzyl, H-6), 9.32 (1H, bs, NH). ^{13}C NMR (400 MHz, CDCl_3) δ : 37.76 (CH_3),
10 63.94 (C-5'), 71.61, 72.10, 76.25, 76.56 (C-2', C-3', C-1'', CH_2), 85.61, 87.68 (C-1', C-4'), 102.12 (C-5), 126.91, 127.17, 127.36, 128.27, 128.67, 135.24 (Ar), 138.31 (C-6), 140.28, 141.20 (Ar), 149.54 (C-2), 162.94 (C-4).

Preparation of benzoate 109 and 5'-alcohol 110

- 15 Nucleoside 108 (1.56 g, 3.11 mmol) was dissolved in dry *N,N*-dimethylacetamide (40 mL) under N_2 , and sodium benzoate (2.25 g, 15.61 mmol) was added. The slurry was heated to 100°C and stirred at this temperature for 3 hours. The mixture was filtered through a thin pad of Celite™, which was washed with plenty of CH_2Cl_2 . The combined filtrates were diluted with CH_2Cl_2 (150 mL) and washed with saturated aqueous NaHCO_3 (3 x 50 mL), dried (Na_2SO_4) and evaporated under reduced pressure. The remaining material was passed through a small column of silica; elution with 0-1 % MeOH in CH_2Cl_2 , v/v, affording a clear colourless syrup. This material was dissolved in a minimum amount of hot 96 % EtOH, and on cooling a white crystalline product formed, which was isolated by filtration and dried under high vacuum, yielding benzoate 109 (1.41 g, 86 %).

An analytical sample of 109 was debenzoylated by treatment with NH_4OH in MeOH and gave the 5'-alcohol 110 as a white powder after recrystallisation from EtOH/water (1:1, v/v). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ : 3.72 (1H, d, J 7.88, H-1''a), 3.81 (2H, d, J 5.31, H-5'a+b), 3.89 (1H, d, J 7.88, H-1''b), 3.97 (1H, s, H-3'), 4.50 (1H, s, H-2'), 4.66 (2H, s, CH_2 , phenylbenzyl), 5.31 (1H, t, J 5.50, 5'-OH), 5.51 (1H, s, H-1'), 5.64 (1H, d, J 8.06, H-5), 7.34-7.45 (m, 5H, phenylbenzyl), 7.48-7.67 (m, 4H, phenylbenzyl), 7.76 (1H, d, J 8.24, H-6), 11.38 (1H, s, NH). ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$) δ : 56.05 (C-5'), 70.89, 71.65, 75.93, 76.56 (C-2',

C-3', CH₂, C-1''), 86.55, 88.38 (C-1', C-4'), 100.95 (C-5), 126.60, 126.68, 127.46, 128.08, 128.96, 137.15 (Ar), 139.07 (C-6), 139.52, 139.91 (Ar), 150.04 (C-2), 163.37.

5 Preparation of 3'-alcohol 111 and mono LNA-U 112

To a stirred solution of nucleoside **109** (910 mg, 1.73 mmol) in dry CH₂Cl₂ (20 mL) under N₂ was added anhydrous FeCl₃ (Aldrich, 99.99 + %, 560 mg, 3.45 mmol). The reaction mixture (initially a clear red-brown solution. After ca. 30 min. a brown precipitate was observed, which changed to green-blue after another 30 min.) was 10 stirred at room temperature for 2.5 hours. The reaction was quenched by addition of water (10 mL) and diluted with CH₂Cl₂. The mixture was filtrated through a thin pad of Celite™, that was washed with CH₂Cl₂ and MeOH. The combined filtrates were washed with saturated aqueous NaHCO₃ (2 × 50 mL), dried (Na₂SO₄) and evaporated under reduced pressure. The residue was purified by column chromatography on 15 silica: packed in 1 % MeOH in CH₂Cl₂, v/v, and eluted with 2 - 5 % MeOH in CH₂Cl₂, v/v, and gave 3'-alcohol **111** (344 mg, 56 % yield) as a white solid material.

An analytical amount of **111** was debenzoylated by treatment with NH₄OH in MeOH and gave LNA-U-diol **112** as a white powder after recrystallization from MeOH.

20 ¹H NMR (400 MHz, DMSO-*d*₆) δ: 3.62 (1H, d, *J* 7.87, H-1''a), 3.75 (2H, bd, *J* 4.39, H-5'a + b), 3.83 (1H, d, *J* 7.87, H-1''b), 3.87 (1H, bd, *J* 2.75, H-3'), 4.14 (1H, s, H-2'), 5.14 (1H, bt, *J* 4.95, 5'-OH), 5.42 (1H, s, H-1'), 5.62 (1H, d, *J* 8.06, H-5), 5.66 (1H..bd, *J* 3.66, 3'-OH), 7.75 (1H, d, *J* 8.24, H-6), 11.34 (1H, bs, NH).). ¹³C NMR (NMR (400 MHz, DMSO-*d*₆) δ: 56.03 (C-5'), 68.71, 71.07, 78.96 (C-2', C-3', C-1''), 86.43, 88.93 (C-1', C-4'), 100.89 (C-5), 139.20 (C-6), 150.04 (C-2), 163.31 (C-4).

Example 12

Preparation of nucleoside **4C**

30 To a stirred suspension of bismesylate **3** (13.0g, 25.47 mmol) and N⁴-acetylcytosine (6.24 g, 40.75 mmol) in dry CH₃CN (250 mL) under N₂ was added *N*,*O*-bis(trimethylsilyl)acetamide (25.0 mL, 102.25 mmol, Fluka 97 %). The mixture was heated to 40 °C and stirred at this temperature until a clear solution resulted (ca. 20 min.). The mixture was cooled to room temperature and trimethylsilyl triflate (10.0

mL, 55.34 mmol) was added dropwise. The resulting reaction mixture was heated to reflux and stirred at this temperature for 2.5 hours. The mixture was cooled in an ice bath and saturated aqueous NaHCO₃ (100 mL) was carefully added. The formed solid material was filtrated off and washed with CH₂Cl₂ (60 mL). Layers were separated

5 and the aqueous layer was extracted with CH₂Cl₂ (100 mL). The combined organic layers were diluted with CH₂Cl₂ (250 mL) and washed with saturated aqueous NaHCO₃ (2 x 100 mL), dried (MgSO₄) and evaporated under reduced pressure. The residue (yellow oil) was subjected to column chromatography on silica: packed in 1 % MeOH in CH₂Cl₂, elution with 1 - 2 % MeOH in CH₂Cl₂, v/v and gave the title

10 compound **4C** (9.16g, 60 % yield) as a pale yellow foam.

¹H NMR (400 MHz, CDCl₃) δ: 2.12 (3H, s, CH₃ (Ac)), 2.26 (3H, s, CH₃ (Ac)), 3.00 (3H, s, CH₃ (Ms)), 3.01 (3H, s, CH₃ (Ms)), 4.35-4.80 (8H, m, H-2', H-3', H-1''a+b, H-5'a+b, CH₂-benzyl), 5.72-5.73 (2H, m, H-1', H-5), 7.27-7.42 (5H, m, Ar), 7.70 (1H, d, J7.50, H-6), 9.50 (1H, bs, NH). ¹³C NMR (400 MHz, CDCl₃) δ: 20.66, 24.86 (2 x CH₃ (Ac)), 37.40, 37.51 (2 x CH₃ (Ms)), 67.67, 68.05, 73.84, 74.35, 77.89 (C-2', C-3', C-5', C-1'', CH₂ (Bn)), 84.62 (C-4'), 94.58 (C-1'), 96.88 (C-5), 128.24, 128.27, 128.47, 136.59 (Ar), 146.72 (C-6), 154.25 (C-2'), 163.19 (C-4), 169.75, 170.59 (2 x CO).

20 Preparation of nucleoside **5C**

Nucleoside **4C** (5.6 g, 9.28 mmol) was dissolved in THF/H₂O (90 mL, 1/1, v/v), and LiOH·H₂O (2.34 g, 55.76 mmol) was added. The reaction mixture was stirred at room temperature for 4 hours and the mixture was concentrated under reduced pressure to ca. 50 mL. The residue was partitioned between CH₂Cl₂ (200 mL) and saturated aqueous NaHCO₃ (100 mL). The bright yellow aqueous layer was extracted with CH₂Cl₂ (2 x 100 mL). To the combined organic layers, containing precipitated product, was added MeOH until a clear solution was obtained, which was dried (Na₂SO₄) and evaporated under reduced pressure. The remaining pale yellow solid material was dried under high vacuum and gave **5aC** (3.75 g, 95 % yield), which was used without further purification. ¹H NMR (400 MHz, CD₃OD) δ: 3.15 (3H, s, CH₃ (Ms)), 3.89 (1H, d, J 8.06, H-1''a), 3.92 (1H, s, H-3'), 4.06 (1H, d, J 7.87, H-1''b), 4.52 (1H, s, H-2'), 4.56 (1H, d, J 11.54, CHaHb (Bn)), 4.60 (1H, d, J 2.93, H-5'a), 4.64 (1H, d, J 2.38, H-5'b), 4.67 (1H, d, J 12.08, CHaHb (Bn)), 5.63 (1H, s, H-1'), 5.89 (1H, d, J 7.51, H-5), 7.28-7.32 (5H, m, Ar), 7.70 (1H, d, J 7.69, H-6). ¹³C

NMR (400 MHz, CD₃OD) δ: 35.53 (CH₃ (Ms)), 64.19, 70.97, 71.32, 75.31, 76.25 (C-2', C-3', C-5', C-1'', CH₂ (Bn)), 84.98 (C-4'), 87.56 (C-1'), 93.88 (C-5), 127.22, 127.32, 127.57, 136.53 (Ar), 139.05 (C-6), 155.61 (C-2), 165.81 (C-4).

5 Preparation of benzoate **5aC** and 3'-alcohol **6aC**

Crude mesylate **5C** (3.75 g, 8.86 mmol) was dissolved in dry DMF (100 mL) under N₂ and sodium benzoate (3.83 g, 26.58 mmol) and Cs₂CO₃ (4.33 g, 13.29 mmol) was added. The suspension was heated to 50°C and stirred at this temperature for 17 hours. The resulting very thick pale yellow slurry was diluted with DMF (100 mL), 10 and more sodium benzoate (2.6 g, 18.04 mmol) was added and the temperature was increased to 65°C. Stirring was continued for 5 hours at this temperature. Then more Cs₂CO₃ (2.2 g) was added and the mixture was stirred for an additional 2.5 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was partitioned between CH₂Cl₂ (250 mL) and 15 saturated aqueous NaHCO₃ (250 mL). The aqueous layer was extracted with CH₂Cl₂ (2 x 150 mL) and the combined organic layers were washed with brine (100 mL), dried (Na₂SO₄) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica: packed in 2 % MeOH in CH₂Cl₂, v/v, elution with 2 - 4 % MeOH in CH₂Cl₂, v/v, and gave benzoate **5aC** (3.35 g, 84 %) as 20 a pale yellow solid material.

To a stirred solution of nucleoside **5aC** (694 mg, 1.54 mmol) in ethanol (15 mL) and cyclohexene (6 mL) was added palladium hydroxide (20 % on carbon moist, 174 mg). The mixture was heated to reflux and stirred at this temperature for 6 hours. 25 More palladium hydroxide (87 mg) and cyclohexene (3 mL) was added and stirring continued at reflux for 17 hours. Then more palladium hydroxide (68 mg) and cyclohexene (2 mL) was added and the mixture was stirred for another 2.5 hours. The reaction mixture was cooled to room temperature and the catalyst was removed by filtration through a small pad of Celite™. The solvents were evaporated under 30 reduced pressure and gave the free 3'-alcohol **6aC** (416 mg, 75 % yield) as a white solid material. ¹H NMR (400 MHz, CD₃OD) δ: 3.96 (1H, d, J 8.97, H-1''a), 4.12 (1H, s, H-3'), 4.13 (1H, d, J 9.00, H-1''b), 4.39 (1H, s, H-2'), 4.73 (1H, d, J 9.83, H-5'a), 4.84 (1H, d, J 9.85, H-5'b), 5.59 (1H, s, H-1'), 5.76 (1H, d, J 7.41, H-5), 7.56-7.72 (3H, m, Ar), 7.75 (1H, d, J 7.45, H-6), 8.07-8.10 (2H, m, Ar). ¹³C NMR

(400 MHz, CD₃OD) δ: 59.27, 69.12, 70.55, 78.80 (C-5', C-3', C-2', C-1''), 85.92, 87.26 (C-1', C-4'), 93.54 (C-5), 128.02, 128.69, 128.98, 132.82 (Ar), 139.04 (C-6), 155.56 (C-2), 165.16 (C-4), 165.74 (CO).

5 Preparation of diol 7aC

Nucleoside 6aC (390 mg, 1.08 mmol, crude material), was co-evaporated with dry pyridine (3x) and re-dissolved in dry pyridine (5.0 mL) under N₂. Benzoyl chloride (0.25 mL, 2.15 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 60 min. The mixture was cooled in an ice bath and MeOH (20 mL) was added followed by addition of 2 M NaOH (5.0 mL). The reaction mixture was stirred at 0-5°C for 20 min., then diluted with CH₂Cl₂ (100 mL) and washed with saturated aqueous NaHCO₃ (2 x 50 mL). The organic layer was dried (Na₂SO₄) and evaporated under reduced pressure. The residue was subjected to column chromatography on silica: packed in 2 % MeOH in CH₂Cl₂, v/v, elution with 5-7 % MeOH/ CH₂Cl₂, v/v, and gave the protected nucleoside 7aC (97 mg, 25 % yield) as a white solid material. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 3.71 (1H, d, *J* 7.69, H-1''a), 3.79-3.82 (2H, m, H-5'a + b), 3.87-3.89 (2H, m, H-1''b, H-3'), 4.24 (1H, s, H-2'), 5.17 (1H, t, *J* 5.67, OH), 5.53 (1H, s, H-1'), 5.68 (1H, d, *J* 7.48, H-5), 7.40-7.65 (3H, m, Ar), 7.99 (2H, d, *J* 7.33, Ar), 8.25 (1H, d, *J* 7.51, H-6), 11.26 (1H, bs, NH). ¹³C NMR (400 MHz, DMSO-*d*₆) δ: 56.31, 68.53, 71.20, 78.69 (C-1'', C-2', C-3', C-5'), 87.50, 89.25 (C-1', C-4'), 96.03 (C-5), 128.52, 132.83 (Ar), 144.31 (C-6), 163.35 (C-4).

Example 13

25

1-(2-O-Acetyl-3-O-benzyl-4-C-methansulfonyloxyethyl-5-O-methanesulfonyl-β-D-ribofuranosyl)-6-N-benzoyladenine (4D)

6-N-Benzoyladenine (11.02 g.; 46.1 mmol) was dried *in vacuo* overnight. 1,2-Di-O-acetyl-3-O-benzyl-4-C-methanesulfonyloxyethyl-5-O-methanesulfonyl-D-ribofuranose (19.6 g.; 38.4 mmol) (3) was coevaporated in anh. acetonitrile (3x50 mL) and dried *in vacuo* overnight. 3 was redissolved in anh. 1,2-dichloroethane (stored over molecular sieves) (175 mL), 6-N-Benzoyladenine was added followed by *N,O*-bistrimethylsilylacetamide (25.1 mL; 101.3 mmol). The mixture was refluxed for 1h and cooled to rt. TMS-triflate (13.9 mL; 76.8 mmol) was added and the mixture

was refluxed for 5h, stirred overnight at rt, refluxed for further 24h (red-brown solution) and cooled to rt. The solution was poured into an ice-cold saturated aqueous solution of NaHCO₃ (200 mL) and stirred for 0.5h. The precipitate was filtered off, the phases were separated and the organic phase was washed with a 5 saturated aqueous solution of NaHCO₃ (3x150 mL), dried (Na₂SO₄) and evaporated. Purification by silica gel column chromatography (1-1.5% MeOH in CH₂Cl₂) gave **4D** as a slightly yellow solid in 68% yield (18.0 g.). NMR was consistent with the data reported in an earlier patent.

10 (1S,3R,4R,7S)-7-Benzylxyloxy-1-methanesulfonyloxymethyl-3-(6-N-benzoyl-adenine-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (5D)

4D (17.9 g.; 26.1 mmol) was dissolved in THF (160 mL) and water (110 mL). LiOH·H₂O (5.5 g.; 131 mmol) was added and the mixture was stirred for 3.5h at rt. The solution was neutralized with AcOH (~6 mL) to give a precipitate. The precipitate 15 was filtered off and washed with water to give **5D** as an off-white solid in 80% yield, (11.6 g.). From the mother liquor was additional **5D** isolated by filtration as a yellow-solid (940 mg.; 6%). δ_H (DMSO-d₆/CDCl₃): 8.63 (1H, s), 8.30 (1H, s), 8.04 (2H, m) 7.53-7.42 (3H, m), 7.25-7.21 (5H, m), 6.10 (1H, s), 4.82 (1H, s), 4.67-4.56 (4H, m), 4.41 (1H, s), 4.11 (1H, d, J = 7.9 Hz), 3.96 (1H, d, J = 8.1 Hz), 3.04 (s, 3H). δ_C 20 (DMSO-d₆/CDCl₃): 165.5, 151.5, 150.8, 150.1, 140.6, 136.6, 133.1, 132.0, 128.2, 128.0, 127.9, 127.6, 127.3, 124.5, 86.0, 84.8, 78.1, 76.6, 71.8, 71.7, 64.7, 37.1.

(1S,3R,4R,7S)-7-Benzylxyloxy-1-benzoyloxymethyl-3-(6-N-benzoyl-adenine-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (28)

(1S,3R,4R,7S)-7-Benzylxyloxy-1-methanesulfonyloxymethyl-3-(6-N-benzoyl-adenine-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (5D) (11.5 g.; 20.8 mmol) was dissolved in anh. DMF (450 mL). Sodium benzoate (5.40 g.; 37.4 mmol) was added and the mixture was heated to 90°C for 7h. The solution was cooled to rt., filtered, evaporated and 30 coevaporated with AcCN. The residue was redissolved in dichloromethane (150 mL) and a saturated aqueous solution of NaHCO₃ (150 mL) was added. The phases were separated and the organic phase was washed with a saturated aqueous solution of NaHCO₃ (2x100 mL) and brine (100 mL), dried (Na₂SO₄) and evaporated to give 12.5 g. of a yellowish solid. Recrystallization from EtOH:H₂O (1250 mL; 1:1 v/v) gave **28**

in 88% yield (10.63 g.). δ_H (DMSO-d₆): 11.2 (1H, br s), 8.72 (1H, s), 8.48 (1H, s), 8.06 (2H, m), 7.94 (2H, m), 7.66 (2H, m), 7.54 (4H, m), 7.36-7.26 (5H, m), 6.11 (1H, s), 4.97 (1H, s), 4.82 (2H, s), 4.77 (1H, s), 4.75 (1H, d, $J=12.4$ Hz), 4.69 (1H, d, $J=11.9$ Hz), 4.19 (1H, d, $J=8.0$ Hz), 4.07 (1H, d, $J=7.9$ Hz). δ_C (DMSO-d₆): 165.3, 150.5, 141.9, 137.7, 133.7, 132.5, 129.3, 129.1, 128.9, 128.6, 128.5, 128.3, 127.7, 127.6, 125.7, 85.9, 85.3, 77.9, 77.1, 72.0, 71.3, 60.6.

(1*S*,3*R*,4*R*,7*S*)-7-Benzylxyloxy-1-hydroxymethyl-3-(adenin-9-yl)-2,5-dioxabicyclo-[2.2.1]heptane (27)

10 **28** (10.6 g.; 18.4 mmol) was suspended in a mixture of MeOH (125 mL) and ammonium hydroxide (250 mL). The solution was stirred overnight at room temperature and more ammonium hydroxide (100 mL) was added. Additional ammonium hydroxide (50 mL) was added after 7h and the mixture was stirred overnight. Additional ammonium hydroxide (50 mL) was again added and the mixture
15 was stirred overnight, filtered and dried to give **27** (6.12 g.; 90%) as an off-white solid. δ_H (DMSO-d₆): 8.19 (1H, s), 8.15 (1H, s), 7.33-7.30 (5H, m), 5.97 (1H, s), 5.19 (1H, t), 4.74 (1H, s), 4.63 (2H, s), 4.36 (1H, s), 3.96 (1H, d), 3.83 (3H, m). δ_C (DMSO-d₆): 156.1, 152.8, 148.6, 138.0, 137.9, 128.3, 127.7, 127.6, 119.1, 88.0, 85.4, 77.3, 77.0, 72.1, 71.3, 56.8.

20

(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(adenin-9-yl)-2,5-dioxabicyclo-[2.2.1]heptane (30)

27 (6.0 g.; 16.2 mmol) was suspended in MeOH (100 mL). Pd(OH)₂-C (2 g) was added followed by ammonium formate (8.2 g; 130 mmol) and the solution was
25 heated to 60°C. After 3h more catalyst (1 g.) was added followed by ammonium formate (2 g). After further 4h, the hot solution was filtered through a thin filter paper and washed with boiling MeOH (500 mL). The catalyst was stirred in MeOH (200 mL) overnight and filtered off and was afterwards boiled in MeOH:H₂O (200 mL; 1:1 v/v). Evaporation gave **30** (~ 4 g.; 88%). δ_H (DMSO-d₆): 8.22 (1H, s), 8.15 (1H, s), 7.30 (2H, br s), 5.89 (1H, s), 5.68 (1H, d, $J=4.2$ Hz), 5.05 (1H, t, $J=5.8$ Hz), 4.41 (1H, s), 4.25 (1H, d, $J=3.7$ Hz), 3.92 (1H, d, $J=7.8$ Hz), 3.82 (2H, m), 3.76 (2H, d, $J=7.9$ Hz). δ_C (DMSO-d₆): 156.1, 152.8, 148.5, 137.9, 119.1, 88.6, 85.4, 79.3, 71.5, 70.0, 56.8.

(1*R*,3*R*,4*R*,7*S*)-3-(6-*N*-Benzoyladenine-9-yl)-1-(4,4'-Dimethoxytrityloxymethyl)-7-Hydroxy-2,5-dioxabicyclo[2.2.1]heptane (31)

30 (~ 4g.; 14.3 mmol) was coevaporated several times with anhydrous pyridine. The compound was resuspended in anhydrous pyridine (70 mL). DMCI (6.78 g.; 20 mmol), NEt₃ (2.8 mL; 20 mmol) and DMAP (44 mg.; 0.36 mmol) was added. After 4.5 h. at rt. TMSCl (9.1 mL; 71.5 mmol) was added. After further 45 min. BzCl (8.3 mL; 71.5 mmol) was added and the mixture was stirred overnight, cooled to 0°C followed by addition of water (18 mL). After 5 min, ammonium hydroxide (25-32% (aq)) (35 mL) was added. The cooling bath was removed and the mixture was stirred 10 for 35 min. and evaporated. The residue was redissolved in dichloromethane (150 mL) and brine (150 mL). The phases were separated and the organic phase was washed with brine (150 mL), dried (Na₂SO₄) and evaporated. Purification (2 times) by silica gel column chromatography (0.5-2.5% MeOH in CH₂Cl₂ with 0.5% NEt₃) gave 15 31 as a slightly yellow solid which was dissolved in dichloromethane (5 mL) and precipitated in rapidly vortexing hexanes (400 mL) and filtered. Yield (7.0 g.; 63% from 27). NMR was consistent with earlier reported data (A.A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen and J. Wengel; *Tetrahedron*, 1998, 54, 3607-3630)

20 **Example 14**

9-(2-O-acetyl-3-O-benzyl-4-C-methanesulfonylmethyl-5-O-methanesulfonyl-β-D-ribofuranosyl)-hypoxantine (4E).

To a mixture of compound 3 (4.65 g, 9.13 mmol) and hypoxantine (1.5 g, 10.9 mmol) in 25 anhydrous 1,2-dichloroethane (45 mL) was added BSA (5.3 mL, 21.8 mmol) and the mixture was refluxed for 1h. Trimethylsilyl triflate (1.8 mL, 10.0 mmol) was added dropwise, the mixture was refluxed for 6h and cooled to ambient temperature. Dichloromethane (50 mL) was added, the solution was washed with saturated aqueous NaHCO₃ (2 x 100 mL), dried (Na₂SO₄), and concentrated under reduced pressure. The 30 residue was purified by column silica gel chromatography (1.4 to 6 % of methanol/dichloromethane as eluent) to yield 4.5 g (84 %) of white solid material consisted of two isomers (ca. 1:4 ratio by ¹H-NMR) which was used for next synthesis without additional purification. For compound 4E:

δ_H (CD₃Cl) 12.83 (1H, br s, NH), 8.32, 7.95 (2H, 2 x s, 8-H, 2-H), 7.40-7.31 (5H, m, Bn), 6.18 (1H, d, J 3.5, 1'-H), 6.00 (1H, dd, J'5.9, J''3.5, 2'-H), 5.03 (1H, d, J 6.0, 3'-H), 4.65 (2H, s), 4.64 (1H, d, J 11.0), 4.47 (1H, d, J 10.6), 4.42 (1H, d, J 10.5), 4.39 (1H, d, J 11.4), 3.03, 2.96 (2 x 3H, 2 s, methanesulfonyls), 2.11 (3H, s, acetyl). δ_C (CD₃Cl) 169.5 (CH₃CO), 5 158.4 (C-6), 148.0 (C-4), 145.8 (C-2), 139.6 (C-8), 136.4, 128.5, 128.4, 128.3, (Bn), 125.4 (C-5), 87.8, 84.2, 77.6, 74.6, 73.8, 67.6, 67.4 (ribose, Bn), 37.6, 37.4 (methanesulfonyls), 20.5 (acetyl).

- (1S,3R,4R,7S)-7-Benzylxyloxy-1-methanesulfonylmethyl-3-(hypoxanthin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (21).**
- To a solution of compound 4E in 1,4-dioxane (80 mL) was added 1M aq. NaOH (80 mL) and the mixture was stirred for 1h. Acetic acid (20 mL) was added, the solution was concentrated under reduced pressure to ca. half of its volume and cooled in ice-bath. The precipitate formed was filtered off, washed with ice-cold water and dried *in vacuo*. Yield: 15 2.4 g (73%) of white solid material consisted of two isomers (ca. 1:10 by ¹H-NMR). For compound 21:
- δ_H (CD₃Cl/DMSO-d₆) 12.31 (1H, br s, NH), 7.92, 7.86 (2H, 2 x s, 8-H, 2-H), 7.32-7.28 (5H, m, Bn), 6.02 (1H, s, 1'-H), 4.75 (1H, s, 2'-H), 4.65 (2H, s), 4.63 (1H, d, J 7.5), 4.60 (1H, d, J 7.2), 4.31 (1H, s, 3'-H), 4.18 (1H, d, J 8.1), 4.01 (1H, d, J 8.1), 3.08 (3H, 2 s, methanesulfonyl). δ_C (CD₃Cl/DMSO-d₆) 156.7 (C-6), 146.6 (C-4), 144.9 (C-2), 136.4, 136.3, 127.9, 127.6, 127.3, (C-8, Bn), 125.0 (C-5), 85.9, 84.7, 77.0, 76.7, 71.8, 71.7, 64.5 (ribose, Bn), 37.1, (methanesulfonyl).

- (1S,3R,4R,7S)-1-Benzoyloxymethyl-7-benzylxyloxy-3-(hypoxanthin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (22).**
- A mixture of compound 21 (two isomers; 1.95 g, 4.36 mmol) and sodium benzoate (0.94 g, 6.54 mmol) in anhydrous DMF (100 mL) was stirred at 80°C for 24 h. The solution was cooled to room temperature, filtrated and concentrated to an oil. The residue was separated by column silica gel chromatography (2 to 3.5 % methanol/dichloromethane as 30 eluent) to give 1.51 g (73 %) of compound 22 as a white solid material.
- δ_H (CD₃Cl) 13.08 (1H, br s, NH), 8.23, (1H, s 8-H), 7.98 (2H, m, Bz), 7.89 (1H, s, 2-H), 7.60 (1H, m, Bz), 7.46 (2H, m, Bz), 7.25-7.23 (5H, m, Bn), 6.05 (1H, s, 1'-H), 4.83 (1H, s, 2'-H), 4.80 (1H, d, J 12.6), 4.68 (1H, d, J 11.9), 4.67 (1H, d, J 12.8), 4.57 (1H, d, J 11.7), 4.28 (1H, d, J 8.2), 4.27 (1H, s, 3'-H), 4.10 (1H, d, J 7.9).

δ_c (CD₃Cl) 165.7 (Bz), 158.8 (C-6), 147.6 (C-4), 145.3 (C-2), 137.2 (C-8), 136.4, 133.4, 129.4, 129.0, 128.5, 128.4, 128.1, 127.7 (Bz, Bn), 125.1 (C-5), 86.6, 85.8, 77.1, 77.0, 72.5, 72.4, 59.6 (ribose, Bn).

5 **(1S,3R,4R,7S)-7-Benzylxyloxy-1-hydroxymethyl-3-(hypoxanthin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (23).**

To a solution of compound **22** in methanol (20 mL) was added 2M NaOH (2 mL) and 15 min later acetic acid (2 mL). The mixture was cooled in ice-bath, the precipitate was filtered off, washed with water and dried *in vacuo*. Yield: 0.69 g (85 %) of

10 compound **23** as a white solid material.

δ_H (DMSO-d₆) 8.16, (1H, s 8-H), 8.06 (1H, s, 2-H), 7.30-7.20 (5H, m, Bn), 5.95 (1H, s, 1'-H), 4.69 (1H, s, 2'-H), 4.63 (2H, s, Bn), 4.28 (1H, s, 3'-H), 3.95 (1H, d, J 7.7), 3.83 (3H, m). δ_c (DMSO-d₆) 156.6 (C-6), 147.3 (C-4), 146.1 (C-2), 137.9 (C-8), 137.3, 128.3, 127.6, 127.5 (Bn), 124.5 (C-5), 88.2, 85.4, 77.0, 72.1, 71.3, 56.7 (ribose, Bn).

15

(1R,3R,4R,7S)-1-(4,4'-Dimethoxytrityloxymethyl)-7-benzylxyloxy-3-(hypoxanthin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (24).

DMT-chloride (0.7 g, 2.07 mmol) was added to a suspension of compound **23** in anhydrous pyridine and the mixture was stirred at 80°C (oil bath) for 2 h. The solution was 20 diluted with ethyl acetate (150 mL), washed with NaHCO₃ (200 mL), water (2 x 200 mL), dried (Na₂SO₄) and concentrated under reduced pressure. The residue was purified by silica gel HPLC (1 to 4 % of methanol/dichloromethane containing 0.5 % of pyridine) to yield 1.02 g (92%) of compound **24** as a white solid material.

δ_H (CD₃Cl) 13.15 (1H, br s, NH), 8.23, (1H, s 8-H), 8.15 (1H, s, 2-H), 7.45 (m, 2H, DMT), 25 7.36-7.12 (12H, m, Bn, DMT), 6.86-6.80 (m, 4H, DMT), 6.07 (1H, s, 1'-H), 4.71 (1H, s, 2'-H), 4.56 (1H, d, J 11.7, Bn), 4.61 (1H, d, J 11.7, Bn), 4.32 (1H, s, 3'-H), 4.03-3.95 (1H, d, J 7.8), 3.95 (1H, d, J 7.8), 3.78, 3.77 (6H, 2 x s, DMT), 3.58 (1H, d, J 10.9), 3.45 (1H, d, J 11.0). δ_c (CD₃Cl) 159.1, 158.5, 147.6, 145.1, 144.2, 137.5, 136.7, 135.3, 135.2, 129.9, 129.8, 128.9, 128.3, 128.1, 127.9, 127.6, 126.9, 125.2, 113.2 (DMT, Bn, hypoxantine), 30 87.3, 86.6, 86.4, 77.2, 72.8, 72.2, 58.4 (ribose, Bn), 55.1 (DMT).

(1R,3R,4R,7S)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-3-(hypoxanthin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (25).

Sodium formate (1.2 g) was added to a mixture of compound **24** (1.02 g, 0.52 mmol) and 35 Pd/C (10%, 0.5 g) in methanol (20 mL). The mixture was refluxed for 20 min, cooled to

room temperature and diluted with dichloromethane (20 mL). The catalyst was filtered off and the filtrate was concentrated under reduced pressure to give a white solid material, which was crystallised from 50% ethanol/water to yield 680 mg (77%) of compound **25**.

δ_H (DMSO-d₆) 8.12, (1H, s 8-H), 8.09 (1H, s, 2-H), 7.43-7.24 (m, 9H, DMT), 6.90 (m, 4H, 5 DMT), 5.97 (1H, s, 1'-H), 4.44 (1H, s, 2'-H), 4.33 (1H, s, 3'-H), 3.97 (1H, d, J 7.5, Bn), 3.91(1H, d, J 7.7), 3.74 (6H, s, DMT), 3.55 (1H, d, J 10.6). δ_C (DMSO-d₆) 158.2, 156.6, 147.3, 146.1, 144.8, 137.1, 135.5, 135.3, 129.8, 127.9, 127.7, 126.8, 124.6, 113.3 (DMT, hypoxantine), 87.1, 85.6, 79.3, 71.8, 70.5, 59.9 (ribose), 55.1 (DMT).

10 (**1S,3R,4R,7S**)-1-Benzoyloxymethyl 7-benzyloxy- -3-(6-chloropurin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (**26**).

Thionyl chloride (2.1 mL, 29 mmol) and DMF (1mL) were added to a solution of compound **22** (1.24 g, 2.62 mmol) in dichloromethane. The mixture was stirred at 30°C (oil bath) overnight, diluted with ethyl acetate (50 mL), washed with NaHCO₃ (sat. aq., 100 15 mL) and water (2 x 50 mL), dried (Na₂SO₄), and concentrated under reduced pressure. A white solid residue was purified by column silica gel chromatography using 0 to 2 % of methanol/dichloromethane as eluent. Yield: 1.2 g (93%) of compound **26** as a white solid material.

δ_H (CD₃Cl) 8.69 (1H, s, 8-H), 8.16 (1H, s, 2-H), 7.95 (2H, m, Bz), 7.62 (1H, m, Bz), 7.46 (2H, m, Bz), 7.25-7.20 (5H, m, Bn), 6.11 (1H, s, 1'-H), 4.93 (1H, s, 2'-H), 4.82 (1H, d, J 12.7), 4.68 (1H, d, J 11.9), 4.65 (1H, d, J 12.7), 4.57 (1H, d, J 11.9), 4.31 (1H, d, J 8.0), 4.26 (1H, s, 3'-H), 4.12 (1H, d, J 8.0). δ_C (CD₃Cl) 165.7 (Bz), 152.0, 151.3, 150.1, 142.4, 136.2, 133.5, 132.0, 129.4, 129.0, 128.5, 128.4, 128.2, 127.7, 86.9, 86.0, 77.1, 76.7, 72.6, 72.4, 59.5.

25

(**1S,3R,4R,7S**)-7-Benzyl-1-hydroxymethyl-3-(adenin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (**27**).

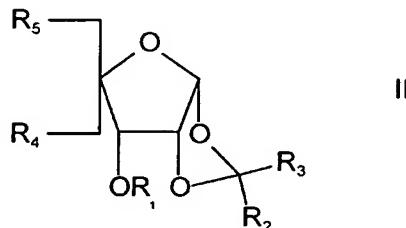
32% NH₄OH (20 mL) was added to a solution of compound **26** (1.2 g, 2.43 mmol) in THF (20 mL) and the mixture was stirred for 48 h at ambient temperature. The solvents were 30 partly removed under reduced pressure (ca. ½ of volume) and the solution was cooled in an ice-bath. The precipitate was collected and re-crystallised from water to yield compound **27** (450 mg, 50.3%) as a white solid material.

δ_H (DMSO-d₆) 8.17, (1H, s 8-H), 8.13 (1H, s, 2-H), 7.30-7.20 (5H, m, Bn), 5.95 (1H, s, 1'-H), 5.15 (1H, t, J 5.1, 5'-OH), 4.72 (1H, s, 2'-H), 4.61 (2H, s, Bn), 4.34 (1H, s, 3'-H), 3.94 (1H, d, J 7.7), 3.81 (3H, m). δ_C (DMSO-d₆) 156.0 (C-6), 152.7 (C-4), 148.6 (C-2), 137.9 (C-

8), 128.2, 127.6, 127.5 (Bn), 119.0 (C-5), 88.0, 85.3, 77.2, 77.0, 72.0, 71.2, 56.8 (ribose, Bn).

CLAIMS

1. A method for the synthesis of a novel intermediate of the general formula II:



5

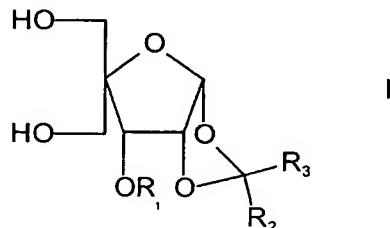
wherein R₁ is selected from optionally substituted aryl(C₁₋₆-alkyl), optionally substituted tetrahydropyran-2-yl, optionally substituted arylcarbonyl and optionally substituted aryl;

each of the substituents R₂ and R₃ is independently selected from hydrogen, optionally 10 substituted C₁₋₆-alkyl, optionally substituted aryl, and optionally substituted aryl(C₁₋₆-alkyl), with the proviso that R₂ and R₃ are not both hydrogen, or R₂ and R₃ together designate C₃₋₇-alkylene; and

each of the substituents R₄ and R₅ independently is R'SO₂O- wherein R' is selected from 15 optionally substituted alkyl and optionally substituted aryl;

said method comprising the following step:

treatment of a compound (hereinafter termed "starting material") of the general formula I:



20

wherein R₁, R₂ and R₃ are as defined above;

with R'SO₂X wherein R' is selected from optionally substituted C₁₋₆-alkyl and optionally substituted aryl, and X designates halogen.

2. A method according to claim 1, wherein R₁ is selected from benzyl, o-, m-, and p-methylbenzyl, 2-chlorobenzyl, 4-phenylbenzyl, tetrahydropyran-2-yl, benzoyl and phenyl.
3. A method according to claim 2, wherein R₁ is benzyl.
4. A method according to any of claims 1-3, wherein each of the substituents R₂ and R₃ independently represent hydrogen methyl, trifluoromethyl, ethyl, propyl, *iso*-propyl, butyl, *t*-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, phenyl, benzyl, phenylethyl, o-, m-, and p-methylbenzyl, 2-chlorobenzyl, or R₂ and R₃ together designate 1,5-pentylene.
5. A method according to any of claims 1-4, wherein each of the substituents R₂ and R₃ independently represent hydrogen, methyl, phenyl, benzyl, phenylethyl, preferably methyl.
6. A method according to any of claims 1-4, wherein each of the substituents R₂ and R₃ are methyl.
7. A method according to claims 1-6, wherein R' is selected from methyl, trifluoromethyl, ethyl, 2,2,2-trifluoroethyl, propyl, *iso*-propyl, butyl, nonafluorobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, benzyl, o-, m- or p-methylbenzyl, 2-chlorobenzyl, phenyl, o-, m- or p-bromophenyl, p-nitrophenyl, and X is selected from halogen, such as fluoro, chloro, bromo, and iodo.
8. A method according to claim 7, wherein R'SO₂X is selected from methanesulfonyl chloride, trifluoromethanesulfonyl chloride, ethanesulfonyl chloride, 2,2,2-trifluoroethanesulfonyl chloride, propanesulfonyl chloride, *iso*-propanesulfonyl chloride, butanesulfonyl chloride, nonafluorobutanesulfonyl chloride, cyclopentanesulfonyl chloride, hexanesulfonyl chloride, cyclohexanesulfonyl chloride, α -toluenesulfonyl chloride, p-toluenesulfonyl chloride, p-bromobenzenesulfonyl chloride and p-nitrobenzenesulfonyl chloride.
9. A method according to claim 8, wherein R'SO₂X is selected from methanesulfonyl chloride, trifluoromethanesulfonyl chloride, ethanesulfonyl chloride, 2,2,2-

trifluoroethanesulfonyl chloride, nonafluorobutanesulfonyl chloride, α -toluenesulfonyl chloride and *p*-toluenesulfonyl chloride.

10. A method according to claim 9, wherein R'SO₂X is selected from methanesulfonyl
5 chloride.

11. A method according to any of claims 1-10, wherein the ratio between compound I and R'SO₂X is in the range of 1:2 to 1:10.

10 12. A method according to claim 11, wherein the ratio between compound I and R'SO₂X is in the range of 1:2-1:5.

13. A method according to claim 12, wherein the ratio between compound I and R'SO₂X is in the range of 1:2-1:4.

15

14. A method according to claim 13, wherein the ratio between compound I and R'SO₂X is in the range of 1:2.5-1:3.5.

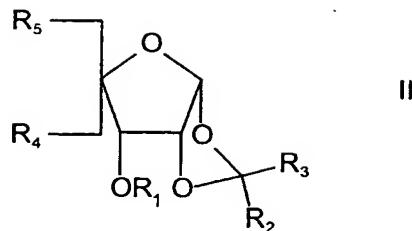
15. A method according to any of claims 1-9, wherein compound I is subsequently treated
20 with two different sulfonyl halides, R^{III}SO₂X and R^{IV}SO₂X, wherein R^{III} and R^{IV} are independently selected from the group of substituents defined for R' provided that R^{III} and R^{IV} do not represent the same group, and X is a halogen.

16. A method according to claim 15, wherein compound I is first treated with R^{III}SO₂X in
25 the ratio 1:1-1:1.5 to afford compound II, wherein R₄ is R^{III}SO₂O- and R₅ is hydroxyl and subsequently, the formed compound II is treated with R^{IV}SO₂X in the ratio 1:1-1:2.5, to afford compound II wherein R₄ is R^{III}SO₂O- and R₅ is R^{IV}SO₂O-.

17. A method according to claims 1-16, wherein the treatment of compound I with the
30 sulfonyl halide is performed in the presence of a base, such as pyridine, 4-dimethylaminopyridine, triethylamine, and sodium hydride.

18. A method according to claim 17, wherein the treatment of compound I with the sulfonyl halide is performed in the presence of pyridine or 4-dimethylamino-pyridine.

19. A method according to claim 18, wherein the treatment of compound I with the sulfonyl halide is performed in the presence of pyridine.
20. A method according to any of claims 1-19, wherein the treatment of compound I with
5 the sulfonyl halide is performed in the presence of a solvent selected from pyridine,
tetrahydrofuran, toluene, xylene, benzene, ether, ethylacetate, acetonitrile, triethylamine,
N,N-dimethylformamide, dimethylsulfoxide, dichloromethane, and 1,2-dichloroethane.
21. A method according to any of claims 17-20, wherein the base and the solvent is
10 constituted by the same substance.
22. A method according to any of claims 1-21, wherein the treatment of compound I with
sulfonyl halide is performed at -30°C to 40°C.
- 15 23. A method according to claim 22, wherein the treatment of compound I with sulfonyl
halide is performed at -5°C to 30°C.
24. A method according to claim 23, wherein treatment of compound I with sulfonyl halide
is performed at preferably 0°C to 25°C.
20
25. A method according to any of claims 1-24, wherein R₁ is benzyl.
26. A method according to any of claims 1-25, wherein R₂ and R₃ are selected from
methyl, ethyl, propyl, *iso*-propyl, butyl, phenyl, benzyl, phenylethyl, or R₂ and R₃ together
25 designate 1,5-pentylene.
27. A method according to any of claims 1-26, wherein R₂ and R₃ both represent methyl.
28. A method according to any of claims 1-27, wherein R₁ represents benzyl and R₂ and
30 R₃ both represent methyl.
29. A compound of the general formula II:



wherein R₁ is selected from optionally substituted aryl(C₁₋₆-alkyl), optionally substituted tetrahydropyran-2-yl, optionally substituted arylcarbonyl and optionally substituted aryl;

- 5 each of the substituents R₂ and R₃ is independently selected from hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted aryl, and optionally substituted aryl(C₁₋₆-alkyl), with the proviso that R₂ and R₃ are not both hydrogen, or R₂ and R₃ together designate C₃- γ -alkylene; and
- 10 each of the substituents R₄ and R₅ independently is R'SO₂O- wherein R' is selected from optionally substituted alkyl and optionally substituted aryl.

30. A compound according to claim 29, wherein R₁ is selected from benzyl, o-, m-, and p-methylbenzyl, 2-chlorobenzyl, tetrahydropyranyl, benzoyl and phenyl.

15

31. A compound according to claim 30, wherein R₁ represents benzyl.

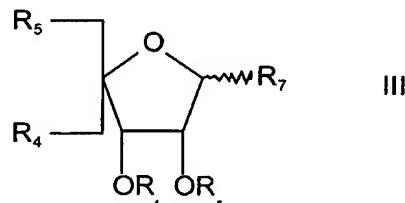
32. A compound according to any of claims 29-31, wherein each of the substituents R₂ and R₃ are independently selected from hydrogen, methyl, trifluoromethyl, ethyl, propyl, iso-propyl, butyl, t-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, phenyl, benzyl, phenylethyl, o-, m-, and p-methylbenzyl, 2-chlorobenzyl, or R₂ and R₃ together are selected from 1,3-propylene, 1,4-butenylene, 1,5-pentylene.

33. A compound according to any of claims 29-32, wherein each of the substituents R₂ and R₃ are independently selected from hydrogen, methyl, phenyl, benzyl, and phenylethyl.

34. A compound according to any of claims 29-33, wherein each of the substituents R₂ and R₃ are methyl.

35. A compound according to any of claims 29-34, wherein each of the substituents R₄ and R₅ are selected from methanesulfonyl, trifluoromethanesulfonyl, ethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, propanesulfonyl, iso-propanesulfonyl, butanesulfonyl,
 5 nonafluorobutanesulfonyl, pentanesulfonyl, cyclopentanesulfonyl, hexanesulfonyl, cyclohexanesulfonyl, α-toluenesulfonyl, 2-chloro-α-toluenesulfonyl, o-, m-, p-toluenesulfonyl, benzenesulfonyl, o-, m-, p-bromobenzenesulfonyl, and o-, m-, p-nitrobenzenesulfonyl.
- 10 36. A compound according claim 35, wherein each of the substituents R₄ and R₅ are methanesulfonyl, trifluoromethanesulfonyl, o-, m-, p-toluenesulfonyl and o-, m-, p-bromobenzenesulfonyl, more preferably methanesulfonyl, and o-, m-, p-toluenesulfonyl,
- 15 37. A compound according claim 36, wherein both of the substituents R₄ and R₅ are methanesulfonyl.

38. A compound of the general formula III:



- 20 wherein R₁ is selected from optionally substituted aryl(C₁₋₆-alkyl), optionally substituted tetrahydropyran-2-yl, optionally substituted arylcarbonyl and optionally substituted aryl; and
 each of the substituents R₄ and R₅ independently is R'SO₂O- wherein R' is selected from
 25 optionally substituted alkyl and optionally substituted aryl; and

R₆ is selected from hydrogen, optionally substituted (C₁₋₆-alkyl)carbonyl, optionally substituted arylcarbonyl, optionally substituted aryl(C₁₋₆-alkyl), optionally substituted C₁₋₆-alkyl, and tri(alkyl/aryl)silyl; and

R₇ is selected from optionally substituted (C₁₋₆-alkyl)carbonyloxy, optionally substituted C₁₋₆-alkoxy, halogen, optionally substituted arylthio, optionally substituted C₁₋₆-alkylthio, and optionally substituted aryloxy.

- 5 39. A compound according to claim 38, wherein R₁ is selected from benzyl, o-, m-, and p-methylbenzyl, 2-chlorobenzyl, tetrahydropyran-2-yl, benzoyl, phenyl,
40. A compound according to claim 39, wherein R₁ is benzyl.
- 10 41. A compound according to any of claims 38-40, wherein each of the substituents R₄ and R₅ are selected from methanesulfonyl, trifluoromethanesulfonyl, ethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, propanesulfonyl, *iso*-propanesulfonyl, butanesulfonyl, nonafluorobutanesulfonyl, pentanesulfonyl, cyclopentanesulfonyl, hexanesulfonyl, cyclohexanesulfonyl, α -toluenesulfonyl, 2-chloro- α -toluenesulfonyl, o-, m-, p-toluenesulfonyl, benzenesulfonyl, o-, m-, p-bromobenzenesulfonyl, and o-, m-, p-nitrobenzenesulfonyl.
- 15 42. A compound according to claim 41, wherein each of the substituents R₄ and R₅ are selected from methanesulfonyl, trifluoromethanesulfonyl, and o-, m-, p-toluenesulfonyl,
- 20 more preferably methanesulfonyl, and o-, m-, p-toluenesulfonyl.
43. A compound according to claim 42, wherein both of the substituents R₄ and R₅ are methanesulfonyl.
- 25 44. A compound according to any of claims 38-43, wherein R₆ represents acetyl, benzoyl, m-trifluoromethylbenzoyl and benzyl.
45. A compound according to any of claims 38-44, wherein R₇ represents acetoxy, methoxy, ethoxy, chloride, fluoride, bromide, iodide and -SC₆H₅.
- 30 46. A compound according to any of claims 38-45, wherein R₁ represents benzyl and R₄ and R₅ are both selected from methanesulfonyl, trifluoromethanesulfonyl, ethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, butanesulfonyl, nonafluorobutanesulfonyl, α -toluenesulfonyl, p-toluenesulfonyl, benzenesulfonyl, p-bromobenzenesulfonyl, and p-nitrobenzenesulfonyl.

47. A compound according to claim 46, wherein R₄ and R₅ both are selected from methanesulfonyl, trifluoromethanesulfonyl, *p*-toluenesulfonyl and *p*-bromobenzene-sulfonyl.
- 5 48. A compound according to claim 47, wherein R₄ and R₅ both are selected from methanesulfonyl, and *p*-toluenesulfonyl.
49. A compound according to claim 48, wherein R₄ and R₅ both represent methanesulfonyl.
- 10 50. A compound according to claim 48, wherein R₁ represents benzyl, R₄ and R₅ represent methanesulfonyl, R₆ represents acetyl, and R₇ represents acetoxy.
51. The use of a compound of the formula II, as defined in any of the claims 29-37, for the
15 preparation of a [2.2.1]bicyclo nucleosides and derivatives thereof.
52. The use of a compound of the formula III, as defined in any of the claims 38-50, for
the preparation of a [2.2.1]bicyclo nucleosides and derivatives thereof.

1/8

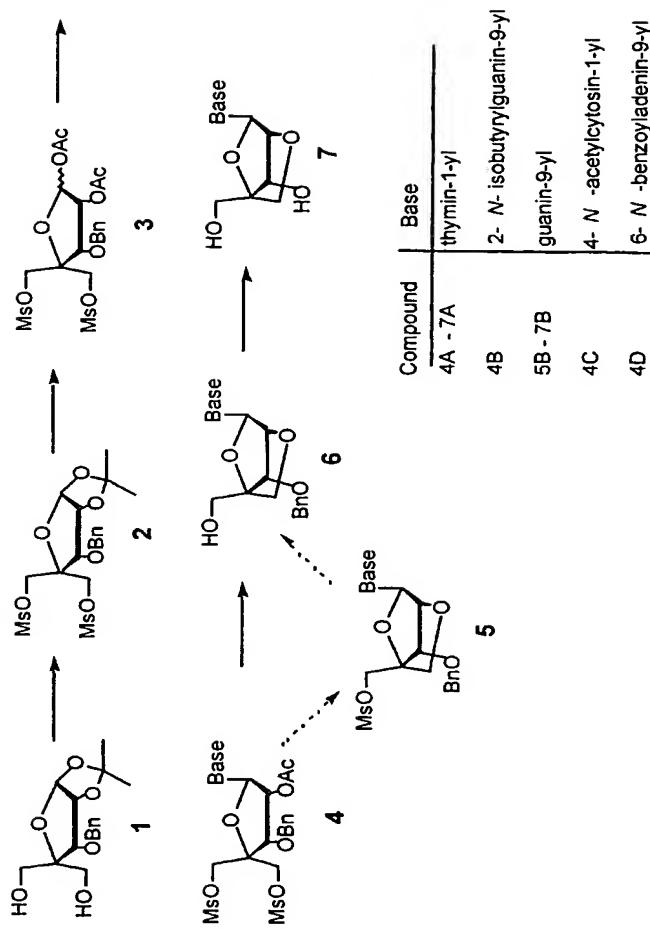


Fig. 1

Fig. 1

2/8

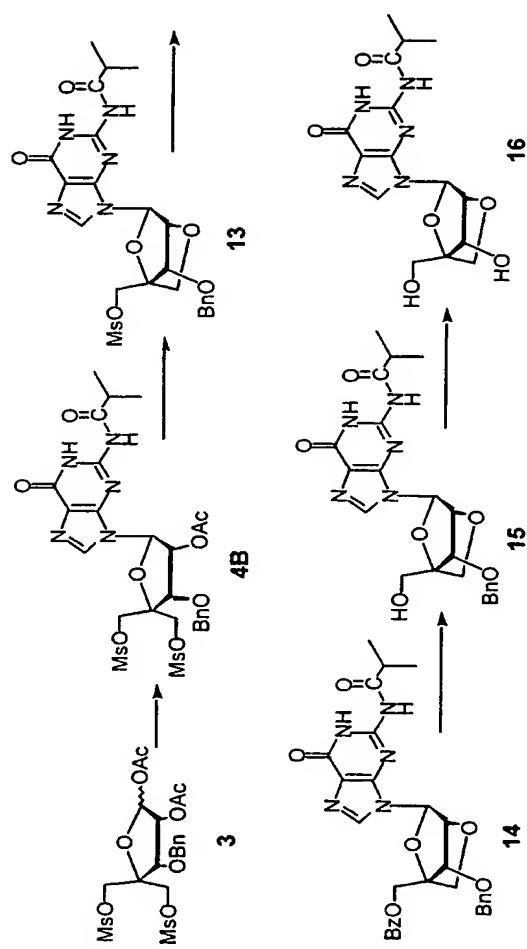


Fig. 2

Fig. 2

3/8

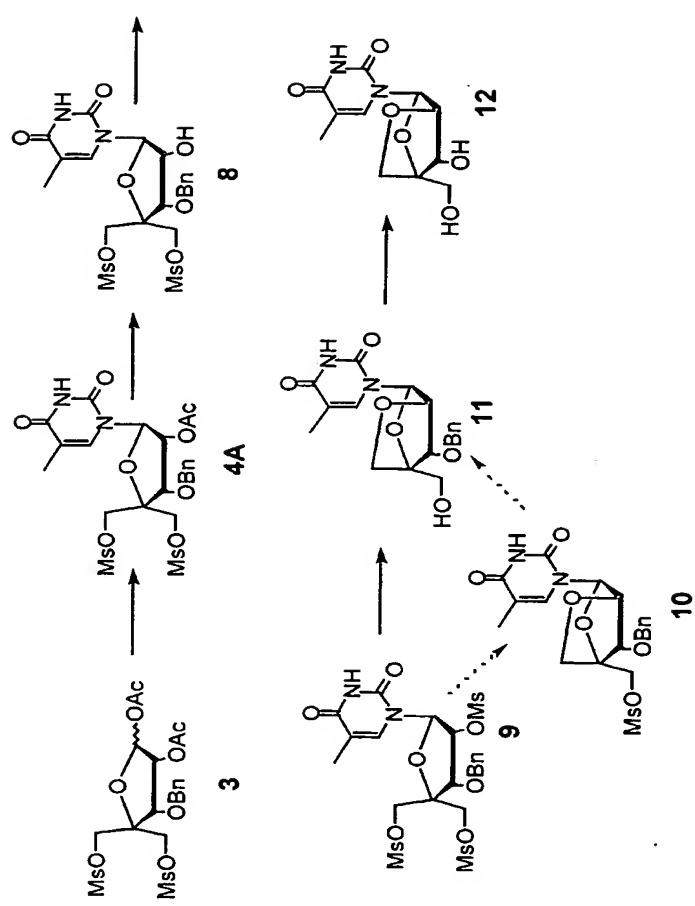


Fig. 3

Fig. 3

4/8

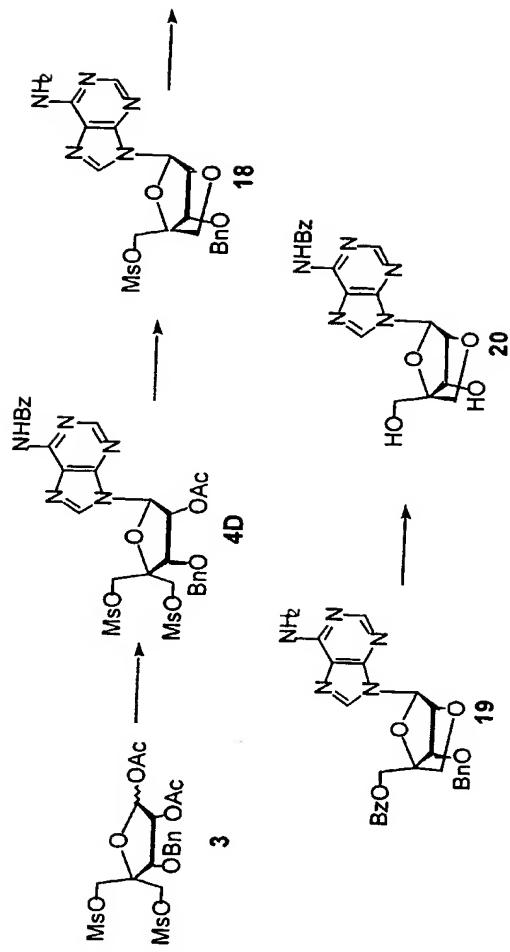


Fig. 4

Fig. 4

5/8

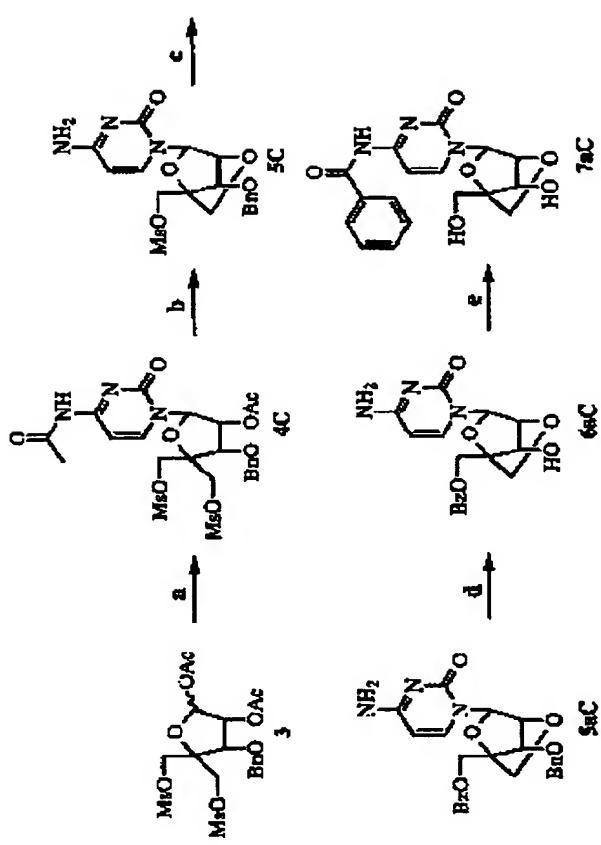


Fig. 5

Fig. 5

6/8

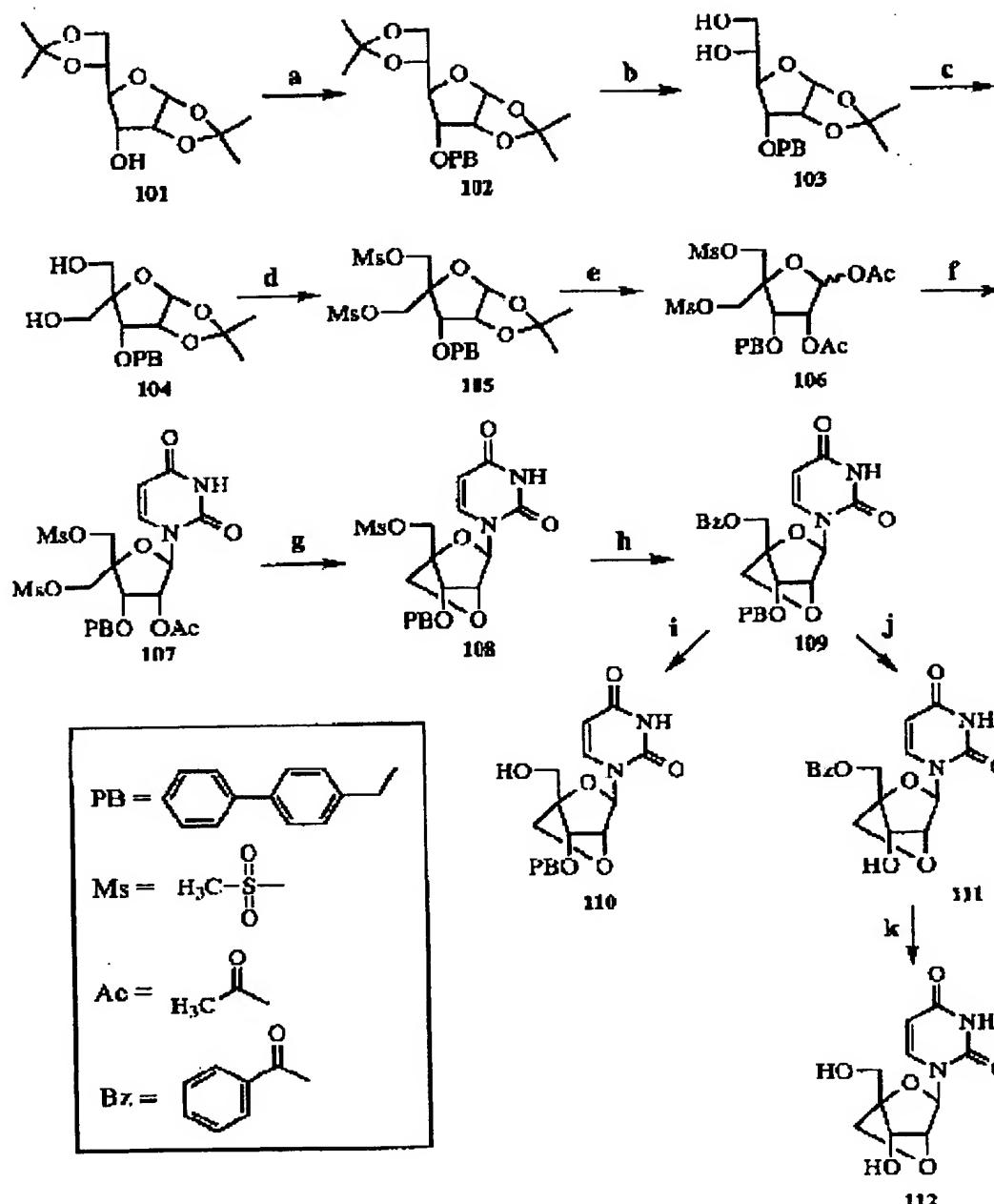
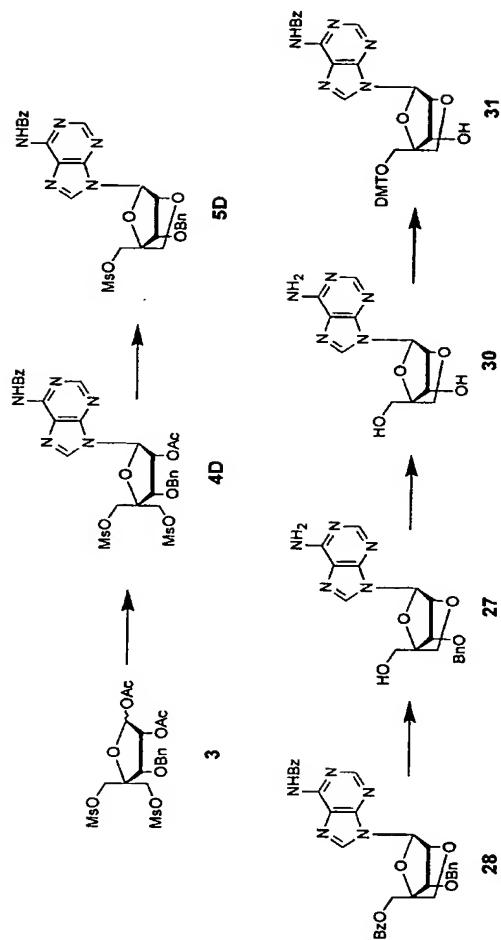


Fig. 6

Fig. 6

7/8

**Fig. 7****Fig. 7**

8/8

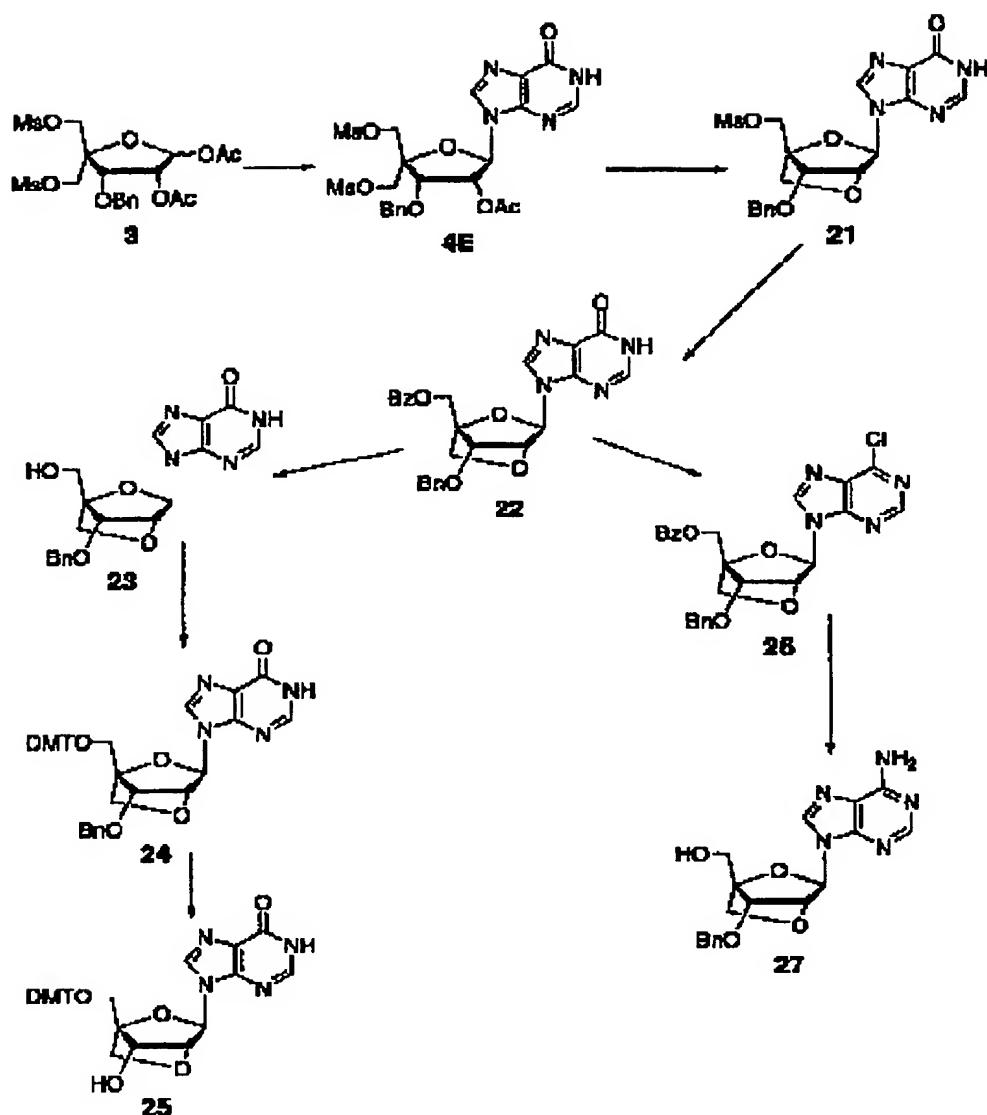


Fig. 8

Fig. 8

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 September 2000 (28.09.2000)

PCT

(10) International Publication Number
WO 00/56746 A3

- (51) International Patent Classification⁷: C07H 9/04, (81) Designated States (*national*): AE, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (21) International Application Number: PCT/DK00/00141
- (22) International Filing Date: 24 March 2000 (24.03.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
PA 1999 00407 24 March 1999 (24.03.1999) DK
PA 2000 00099 21 January 2000 (21.01.2000) DK
- (71) Applicant (*for all designated States except US*): EXIQON A/S [DK/DK]; Bygstubben 9, DK-2950 Vedbæk (DK).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): KOCHKINE, Alexei [DK/DK]; c/o Jørgensen, Borgergade 26, apartment 36, DK-1300 Copenhagen K (DK). FENSHOLDT, Jef [DK/DK]; Ærtemarken 79, DK-2860 Søborg (DK). PFUNDHELLER, Henrik, M. [DK/DK]; Dalgas Have 2, 4. tv., DK-2000 Frederiksberg (DK).

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

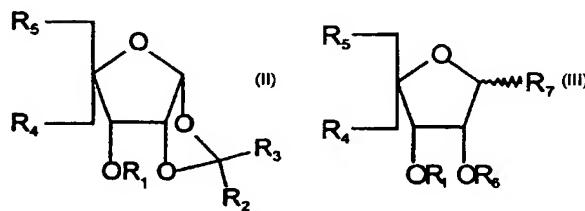
— with international search report

(88) Date of publication of the international search report:
18 October 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IMPROVED SYNTHESIS OF [2.2.1]BICYCLO NUCLEOSIDES

WO 00/56746 A3



fonation of the formed [2.2.1]bicyclo nucleoside, a short route to each the LNA (Locked Nucleic Acid) derivatives of adenosine, cytosine, uridine, thymidine and guanine is demonstrated. The use of the 5'-sulfonated ring-closed intermediate also allows for synthesis of 5'-amino- and thio-LNAs.

(57) Abstract: A synthesis of [2.2.1]bicyclo nucleosides which is shorter and provides higher overall yields proceeds via the key intermediate of general formula (III), wherein R₄ and R₅ are, for instance, sulfonates and R₇ is, for instance, a halogen or an acetate. From compounds in general formula (II), such as 3-O-aryl-4-C-hydroxymethyl-1,2-O-isopropylidene- α -D-ribofuranose, intermediates of general formula (III) are suitable for coupling with silylated nucleobases. Upon one-pot base-induced ring-closure and desul-

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 00/00141

A. CLASSIFICATION OF SUBJECT MATTER	IPC 7 C07H9/04 C07H15/203 //C07H19/06, C07H19/16, C07H21/00
-------------------------------------	---

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TIM FAT TAM ET AL: "A synthetic Route to 4-C-methyl-xylo-furanose" CAN. J. CHEM., vol. 57, 1979, pages 2818-2822, XP002901162 the whole document ---	1-37
X	NIELSEN P ET AL: "Synthesis and chemoselective activation of phenyl 3,5-di-O-benzyl-2-O,4-C-methylene-1-thio-beta-D-ribofuranoside: a key synthon towards alpha-LNA" CHEM. COMMUN., 1998, pages 2645-2646, XP002901163 the whole document	1-28, 38-52
A	---	29-37

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2001	05.04.01
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer Eva Johansson

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 00/00141

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NIELSEN P ET AL: "A new synthetic approach towards alpha- and beta-LNA (locked nucleic acids)" NUCLEOSIDES & NUCLEOTIDES, vol. 18, no. (4&5), 1999, pages 701-702, XP002901164 the whole document	1-28, 38-52
A	---	29-37
P,X	WO 99 14226 A (EXIQON A S) 25 March 1999 (1999-03-25) the whole document	1-52
A	SINGH S K ET AL: "LNA (locked nucleic acids): synthesis and high-affinity nucleic acid recognition" CHEM. COMMUN., 1998, pages 455-456, XP002094303 the whole document	1-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00141

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT,
no additional fees are to be refunded.

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-37

Invention concerning a method for synthesis of a compound with formula II and a compound with formula III

2. Claims: 38-52

Invention concerning a compound with formula III and the use thereof

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/DK 00/00141

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9914226 A	25-03-1999	AU 9063398 A	CN 1279687 T	05-04-1999 10-01-2001

THIS PAGE BLANK (USPTO)